BIOFOULING AND SHORT-TERM DYNAMICS ON FISH CAGE NETTING IN RELATION TO FISH REARING AND ENVIRONMENTAL FACTORS

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STATEMENT

The work presented in this thesis is, to the best of my knowledge and belief, original, except as acknowledged in the text, and the material has not been submitted, either in whole or in part, for a degree at this or any other University.

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ABSTRACT

Four studies were conducted in a floating net-cages farm at Matang Mangrove Forest Reserve (MMFR), Perak, Malaysia, to elucidate the biofouling assemblages on cage nettings and factors that influence their development. In the first study (Chapter 3), biofouling on 1.6 cm mesh net panels (size 0.2 m x 2 m) suspended inside (homemade pellet 'P', trash fish 'T') and outside (O) experimental net-cages in a fish farm were monitored every week until net openings were completely occluded by macrofouling organisms. Eight species (7 phyla) of sessile organisms and 27 species (3 phyla) of non-sessile associates were recorded. Colonization by macrofouling organisms usually began with *Plumularia* sp. and *Gammaropsis* sp. while other species only appeared after 1 or 2 weeks of net panels immersion. Inside net-cages where water flow was slow (mean < 6 cm s^{-1}), macroalgae (*Polysiphonia* sp.), anthozoans (unidentified anemones), barnacles (*Balanus amphitrite*), amphipods (*Gammaropsis* sp. & *Photis* sp.) and tanaids (*Leptognathia* sp.) were dominant on the net panels during the dry season. However, in the wet season, hydroid (*Plumulari*a sp.), mussel (*Xenostrobus mangle*), nematode and copepods (*Euterpina acutifrons*) abundance was significant. With stronger water flow (mean ≈ 20 cm s^{-1}) as occurring outside net-cages, macrofouling assemblages for both seasons comprised mainly *Plumularia* sp. and *Gammaropsis* sp.. The macrofouling assemblage showed a clear succession of species that occupied different depths of the net panels.

It was hypothesized from the first study (Chapter 3) that the use of high quality fish pellet feed should reduce feed wastage and thus biofouling assemblages. However, as revealed in the second study (Chapter 4), the biomass of sessile macrofoulers and their non-sessile associates on net panels inside the net-cages given the high quality feed (commercial pellet 'M') was not significantly ($P > 0.05$) different to that given low quality fish feed (homemade pellet 'P' & trash fish 'T'). These results do not support the proposed hypothesis that a high quality fish feed could help to reduce biofouling assemblages on nets. A reduced flow rate to less than 10 cm s^{-1} inside the net-cage will significantly encourage the rapid development of sessile biofouling biomass (g m^{-2} wk^{-1}), with (175 to 231% higher in treatments M, P & T) or without (56 to 145% higher in treatments N) fish rearing and feed input compared to swifter water flow i.e. >25 cm s^{-1}

outside net-cages (C). However, non-sessile organisms were more attracted to the organic inputs from fish rearing inside the net-cages (i.e. whether M, P & T); their biomass (g m^{-2} wk^{-1}) were 459 to 802% higher compared to treatments without fish rearing and feed input (N) or outside net-cages (C). The physical presence of the net-cage units and net biofouling play a significant role in flow attenuation. However, there was no significant ($P > 0.05$) effect on biofouling due to net-cage position in the fish farm suggesting that the water flow regime within the cage farm was rather consistent.

It was also hypothesized from the first study (Chapter 3) that the higher salinity during the dry season favoured marine and euryhaline forms and thus increases biofouling rates. However, as revealed in the third study (Chapter 5), survival and development of macrofouling assemblages were not solely influenced by salinity but a combination of salinity as well as other abiotic and biotic factors.

The fourth study (Chapter 6) revealed that concentration of dissolved nutrients and chlorophyll-*a* was relatively higher inside the net-cages (pellet 'P'& trash fish 'T') than outside it (O), suggesting that fish rearing and fish feed input contribute to nutrient and phytoplankton enrichment of culture waters. This finding is consistent with findings from the first study (Chapter 3) where population and total wet biomass of macrofoulers were found to be significantly ($P < 0.05$) higher inside the net-cages (P, T) than outside it (O).

From the study, it is recommended that, in order to reduce biofouling rates and improve water quality, floating fish cage farms in tropical estuaries should be carefully sited (to receive maximum water flow) and re-designed (to improve water flow rates). Furthermore, the rearing of fish fingerling which requires fine-mesh (1.6 cm) should preferably begin during the wetter months and located around the farm's periphery so as to reduce biofouling and net clogging.

ABSTRAK

Empat kajian telah dijalankan disangkar penternakan ikan terapung dikawasan Hutan Simpan Bakau Matang, Perak, Malaysia, bagi melihat bagaimana pembentukan biofouling pada jaring sangkar dan faktor mempengaruhi perkembangan biofouling tersebut. Bagi kajian pertama (Bab 3), perkembangan biofouling pada jaring bersaiz mata 1.6 cm (berukuran 0.2 m x 2 m) yang diletakkan didalam (pellet buatan sendiri 'P', ikan baja 'T') dan diluar (O) sangkar kajian diladang penternakan ikan diperhatikan setiap minggu sehingga mata jaring dilitupi sepenuhnya oleh organisma biofouling. Tujuh species (7 filum) organisma sessile dan 27 species (3 filum) organisma bukan-sessile direkodkan. Kolonisasi organisma macrofouling biasanya bermula dengan *Plumularia* sp. dan *Gammaropsis* sp. sementara species lain hanya muncul selepas 1 atau 2 minggu selepas jaring diletakkan dalam sangkar. Didalam sangkar dimana arus airnya perlahan (purata < 6 cm s^{-1}) makroalga (*Polysiphonia* sp.), anthozoan (anemones tidak dicam species), teritip (*Balanus amphitrite*), amphipod (*Gammaropsis* sp. & *Photis* sp.) dan tanaid (*Leptognathia* sp.) dominan pada jaring semasa musim kemarau. Walaubagaimanapun, semasa musim tengkujuh, kelimpahan hydroid (*Plumulari*a sp.), kupang (*Xenostrobus mangle*), nematode dan copepod (*Euterpina acutifrons*) adalah signifikan. Dengan arus air yang deras (purata ≈ 20 cm s^{-1}) sepertimana berlaku diluar sangkar, kelompok makrofouling bagi kedua-dua musim adalah terutamanya ialah *Plumularia* sp. dan *Gammaropsis* sp.. Kelompok makrofouling menunjukkan kelangsungan hidup species yang jelas serta menduduki kedalaman berbeza pada jaring tersebut.

Kajian pertama (Bab 3) membawa kepada hipotesis bahawa penggunaan makanan ikan berkualiti tinggi boleh mengurangkan bahan buangan dan seterusnya pembentukan biofouling. Walaubagaimanapun, kajian kedua (Bab 4) menunjukkan bahawa biomas bagi organisma sessile dan bukan-sessile pada jaring yang diberi makanan berkualiti tinggi (komersial pellet 'M') tidak berbeza secara signifikan ($P > 0.05$) dengan sangkar yang diberi makanan berkualiti rendah (pellet buatan sendiri 'P' & ikan baja 'T'). Keputusan ini tidak menyokong hipotesis bahawa makanan berkualiti tinggi boleh membantu mengurangkan pembentukan biofouling pada jaring. Kadar arus air perlahan kurang dari 10 cm s^{-1} melalui sangkar akan menggalakkan perkembangan signifikan organisma biofouling sessile biomass (g m^{-2} $minggu^{-1}$) (175 – 231% lebih tinggi dalam M, P & T) atau tanpa (56 – 145% lebih tinggi dalam sangkar tanpa ikan, N) kekayaan

organik daripada aktiviti penternakan ikan berbanding dengan arus deras i.e. >25 cm s^{-1} diluar sangkar 'C'. Walaubagaimanapun, organisma bukan-sessile lebih tertarik kepada kandungan organik hasil dari penternakan ikan dalam sangkar (i.e. samada M, P & T); kadar biomass (g m^{-2} $minggu^{-1}$) mereka adalah 459 – 802% lebih tinggi berbanding dengan tanpa ternakan ikan serta pemberian makanan (N) atau diluar sangkar (C). Sangkar ikan dan biofouling pada jaring sangkar ikan tersebut memainkan peranan yang signifikan mengurangkan arus air. Tiada kesan yang signifikan ($P > 0.05$) kepada biofouling disebabkan oleh kedudukan sangkar diladang ternakan ikan tersebut, menunjukan bahawa arus air diseluruh kawasan ternakan adalah selaras.

Ia juga telah dihipotesiskan daripada kajian pertama (Bab 3) bahawa saliniti tinggi semasa musim kemarau sesuai bagi marin dan euryhaline species dan seterusnya meningkatkan kadar biofouling. Walaubagaimanapun, seperti yang ditunjukan dalam kajian ketiga (Bab 5), kelangsungan hidup dan perkembanagn makrofouling bukan hanya dipengaruhi oleh saliniti tetapi kombinasi saliniti, lain-lain faktor biotik dan abiotik.

Kajian keempat (Bab 6) menunjukkan bahawa konsentrasi nutrient terlarut dan klorofil-*a* adalah tinggi didalam sangkar ikan (pellet buatan sendiri 'P' & ikan baja 'T') berbanding diluar sangkar (O), ini mencadangkan bahawa penternakan ikan serta pemberian makanan turut menyumbang kepada kekayaan nutrient dan plankton dalam air pengkulturan. Penemuan in selaras dengan penemuan kajian pertama (Bab 3) dimana populasi dan jumlah berat biomass basah makrofouling didapapti signifikan ($P < 0.05$) tingginya didalam sangkar (P, T) berbanding diluar sangkar (O).

Daripada kajian ini, dicadangkan bahawa bagi mengurangkan kadar biofouling serta memperbaiki kualiti air, sangkar ikan dikawasan muara sungai di tropika haruslah diteliti lokasinya (bagi mendapat arus air maksimum) dan direka semula (bagi meningkatkan kadar arus air). Seterusnya anak ikan yang memerlukan sangkar bermata halus (1.6 cm) digalakkan bermula pada musim tengkujuh serta diletak dibahagian tepi sisi ladang ternakan bagi memperolehi aliran arus air yang maximum supaya kadar biofouling dan penutupan mata jaring dapat dikurangkan.

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CHAPTER 1

GENERAL INTRODUCTION

1.1. Overview of Biofouling

Biofouling is commonly referred to as growth of organisms on a man-made artificial structure that cause negative impacts. Biofouling is also commonly used to distinguish the assemblages of organisms that grow on artificial structures from those that occur on natural objects such as rocks, stones, and mangroves trees. However, some authors refer to the occurrence of epiphytes and epizoans on aquatic living organisms as biofouling or epibiosis since it maybe detrimental to the host (Mouritsen & Bay, 2000).

Biofouling is considered ubiquitous in the aquatic environment and it is a worldwide problem particularly in marine waters (Callow & Callow, 2002). Biofouling is considered a port's phenomenon, being associated with ships, inshore waters and civil engineering structures. Fouling on the hulls of marine vessels has been shown to reduce speed and increase propulsive fuel consumption (Bohlander & Haslbeck, 1990). The damage of boats, ships, port facilities and other marine structures caused by boring and biofouling organisms has been a problem since the beginning of maritime activities in the 4^{th} or 5^{th} century BC (WHOI, 1952; Benson et al., 1973 quoted in Kerr et al., 1998). Aristotle was reported to have stated that small 'fish' which really were barnacles, were able to slow down the ship (Abdul Azis et al., 2001). Biofouling incurs yearly losses of over US $6.5 billion to the global shipping industry mainly from higher fuel consumption and regular maintenance involving cleaning and painting of ship hulls (Adkins et al., 1996; Abdul Azis et al., 2001; Callow & Callow, 2002).

Although the occurrence of biofouling is much related to aqueous environment, many published works focused more on seawater compared to freshwater biofouling. This suggests that biofouling in seawater is a more general problem than in the

freshwater environment. There are relatively few published works on freshwater biofouling (Billard, 1978; Beveridge, 1987), and the diversity of causal organisms is considered lower than in marine waters (Cheah & Chua, 1979; Venugopalan & Wagh, 1990; Madin et al., 2009).

The study of biofouling in the marine environment as done by many researchers has provided various interpretations depending on the scope of their research. Venugopalan & Wagh (1990) concluded that biofouling occurs as a result of settlement and growth of sedentary and semi-sedentary organisms on artificial structures placed in water. Kingsbury (1981) defined marine biofouling as a collective term for organisms growing on artificial structures placed in marine and estuarine environments. Abdul Azis et al. (2001) defined biofouling as the attachment and subsequent growth of visible plants and animals on structures exposed to seawater environment, while Baretta-Bekker et al. (1992) indicated biological fouling or biofouling as growth of sessile algae and animals, especially on a ship's bottom or other artificial underwater structures. Many artificial and natural surfaces upon exposure to seawater become rapidly colonized by marine organisms which secrete a variety of adhesive materials thus causing biofouling (Sutherland, 1980; Lindner, 1984; Cooksey & Cooksey, 1986; Young et al., 1988; Abu et al., 1991; Fletcher et al., 1991; Neu & Marshall, 1991; Hoagland et al., 1993).

Around the world, there are more than 2000 species of biofouling organisms thought to exist on the surfaces of artificial structures including 50 species of bacteria, 110 species of diatoms, 450 species of algae and 1900 species of other animals (Hutchins, 1952 cited in Cook, 2001). The latest finding by Anderson & Hunter (2000) increased the number of biofouling organisms to more than 4000 species worldwide. However, the number of biofouling organisms recorded represents only a very small proportion of the known marine species because only organisms with the ability to adapt to new situations created by man can adhere firmly enough to avoid being

washed off or tolerate the wide fluctuations in environmental conditions (Yebra et al., 2004; Madin et al., 2009).

According to Callow & Callow (2002), biofouling development is a highly dynamic process; the causal organisms and their development depend on the geographical location, season, abiotic and biotic factors. In biofouling investigation lasting one in the coastal waters off Bombay, Venugopalan & Wagh (1990) found as many as 100 species. In Malaysia, Cheah & Chua (1979) recorded more than 34 species of fouling organisms encrusting floating net-cages which were dominated by tunicates, mussels, oysters and algae. In the vicinity of desalination and power plants in the Arabian Gulf coast, Abdul Azis et al. (2001) encountered 31 species of biofouling organisms which exhibited a widely varying pattern of incidence, abundance and succession. A total of 103 biofouling organisms were recorded in a 3-year study of artificial surfaces deployed within the Port of Darwin (Marshall et al., 2003).

In general, biofouling organisms are intertidal and sublittoral species that are commonly observed on rocky shorelines (Cook, 2001). The biofouling organisms can be categorized as microfouling and macrofouling organisms. Microfouling organisms include algal spores, diatoms and marine bacteria that form the primary organic film while the macrofouling organisms include multicellular organisms that develop and overgrow the community of microfouling (Fergusson Wood, 1950). The common macrofouling organisms are tubeworms, barnacles, bryozoans, algae, hydroids, mussels, oysters and many others (Madin et al., 2009).

Biofouling organisms exhibit certain characteristics that enable them to readily colonize artificial structures such as a free-swimming larva phase and a sedentary adult form that can firmly adhere to the substratum with the ability to extract dissolved nutrients or particulate material from the water column. Examples include barnacles,

bivalves, hydroids, bryozoans, anemones and macroalgae. Many non-sessile organisms are able to colonize artificial structures and reside within the sedentary biofouling community; these include echinoderms, isopods, amphipods, errant polychaetes, crabs, shrimps and pycnogonids (Claereboudt et al., 1994; Cook, 1999; Madin et al., 2009).

1.1.1. Biofouling Settlement and Development

The settlement process is an event in the colonization sequence of biofouling while development occurs with further build up of settled biofouling organisms. The settlement and development of biofouling are more complicated in seawater than in freshwater because of broad factors such as salinity and species richness. Marine organisms are known for their remarkable adhesive properties, forming very strong bonds to a variety of surfaces ranging from boat hulls to rocks and to other organisms under a wide range of water parameter conditions (Callow & Callow, 2002). The settlement and development occurs in group or colonies. According to Callow et al. (1997) fouling organisms such as barnacles and *Enteromorpha* have developed strategies to settle in group or gregarious settlement.

Settlement and development processes of biofouling are influenced by both biotic and abiotic factors. The abiotic factors are the measurable environmental parameters such as temperature, salinity, dissolved oxygen, turbidity, current flow, light, and pH, which change according to location and season. Abiotic factors may influence the formation of conditioning, primary film, settlement and build up process of biofouling organisms. The biotic factors that influence biofouling include recruitment rates, competition for space and food, predation and several other disturbances which occur after the settlement (Richmond & Seed, 1991; Madin et al., 2009).

The physical and chemical characteristics of substrate are also important in determining biofouling settlement and development. Biofouling organisms have

different tendency to adhere onto different types of substrate. The roughness, structural colour, chemical composition, surface chemistry, surface energy and wettabbility will affect settlement (e.g. Fletcher & Baier, 1984; Crisp et al., 1985; Rittschoff & Costlow, 1989; Roberts et al., 1991; Becker, 1993; Callow & Fletcher, 1994; Roscoe & Walker, 1995; Holm et al., 1997; O'Connor & Richardson, 1998; Becker et al., 2000). Other factors such as amount of suspended particles, tides, color of seawater and pollution were also thought to influence the settlement and development of biofouling (e.g. Baynes & Szmant, 1989; Mayer-Pinto & Junqueira, 2003; Yan et al., 2006; Madin et al., 2009)

Settlement and development are major factors in determining the fouling community structure. Fouling organisms need a suitable place to settle and adhere before they can complete their life cycle. Settlement and development are therefore considered to be the most important stages in the life cycle of fouling organisms (Hadfield, 1986; Walters et al., 1999; Madin et al., 2009; 2010). On the other hand, these stages are the important considerations to develop effective antifouling materials for biofouling prevention (Finlay et al., 2002).

Traditionally, settlement and development process of biofouling has been considered to consist of four general stages: 1) formation of biochemical conditioning, which includes attachment of organic molecules to the surface or commonly known as conditioning, 2) bacterial colonization, the attachment of microbial cells mainly bacteria in the first stage, 3) the attachment of unicellular organisms including replication of the cells and production of extracellular polymeric substances (EPS) and other metabolites, and 4) the death of some cells, replication, sloughing of biofilm parts and development of more advanced organisms mainly by multicellular forms or macrofouling (Wahl, 1989).

1.1.1.1. Biofilms and Other Microfoulers

Every surface immersed in the water column including those of organisms rapidly becomes coated with biofilm. The biofilm is a very successful form of biofouling and is capable of adapting to extreme environmental conditions such as temperature, salinity, pH etc. Its formation is ubiquitous and there is no surface that cannot be colonized under suitable condition. Organic film accumulation composes of chemical compounds (mostly protein, proteoglycans and polysaccharides) that make the surface suitable for bacterial colonization (Abarzua & Jakubowski, 1995). According to Relman et al. (1990) and De Bernardis et al. (1997), biofilms are heterogeneous in structure, which give advantages for the attachment of several microorganisms. Biofilms function as pioneer communities that developed during the first stage of succession on the substratum. These communities act as attractant and actual substrate where algae and animals subsequently settled (Neal & Yule, 1994; Corner et al., 2007).

Microfouling organisms such as diatoms and cyanobacteria are known to attach and grow on the biofilm and initiate the formation of a mucilaginous or mainly polysaccharide-based biofilm (Wahl, 1989; Cooksey & Wigglesworth-Cooksey, 1995; Beveridge et al., 1997; Stoodley et al., 1999; Baum et al., 2002). The biofilm forms the interface between substratum and water column. Therefore it influences the settlement and succession of benthic invertebrates and macroalgae (Wahl, 1989), and provides food resources (Underwood, 1984; Hawkins et al., 1989; Edgar & Shaw, 1995). In tropical waters, biofilm is mostly made of diatoms and algae while in temperate waters bacteria represent the important component of primary biofilm (Nair & Thampy, 1980). The biofilm layer often enhances settlement of algal spores, larvae of sessile and sedentary invertebrates ranging from cnidarians to bryozoans, and even motile echinoderms and decapod crustaceans (Tamburri et al., 1992; Leitz & Wagner, 1993; Johnson & Sutton, 1994; Abarzua & Jakubowski, 1995; Weber &

Epifanio, 1996; O‘Connor & Richardson, 1998; Suzuki et al., 2001).

1.1.1.2. Macroorganisms as Biofouling Climax Stages

From several days to a week following substrate immersion, multicellular fouling or commonly referred as macrofouling community may develop and overgrow the microfouling community. Macrofouling occurs with the presence of invertebrate planktonic larvae and algal spore settlement (Evans, 1981; Hadfield, 1986; Butman, 1987). The macrofouling organisms include sessile, sedentary, semisedentary and mobile community which develops on to the substrate (Madin et al., 2009). Macrofouling community of several taxa can be divided into two types of either calcareous or 'hard fouling' and noncalcareous or 'soft fouling' (Callow, 1999). Soft fouling comprises algae and invertebrates, such as soft corals, sponges, anemones, tunicates and hydroids. Hard fouling comprises invertebrates such as barnacles, mussels, bryozoans, mussels, oysters and tubeworms. Both groups can cause different degrees of problem to the substratum they occupied. Macrofouling community tends to be more sensitive to the environmental or biological factors than microfouling community.

1.1.2. The Effects of Biofouling Growth

Biofouling affects human interest in different ways depending on their stage of development, species type, geographical location and the type of structure they occupied. The best known are the negative impacts in connection with the shipping and aquaculture industries. The effects of biofouling start as soon as it begins to colonize the substratum, whereas each stage and species causes different degrees of effects. In certain situations the biofouling organisms can be manipulated as useful tools for various functions in the ecological system.

1.1.2.1. Negative Impacts of Biofouling

To the maritime industries, biofouling is the single biggest factor affecting the operation, maintenance and quality of performance of equipment and infra-structure (Anonymous, 2003). The infrastructure or equipment ranging from very small to mega-size scale will encounter the biofouling problem when they are in contact with water particularly seawater. The uneven growth of *Enteromorpha* on port structures where it is customary to dock a ship can make manoeuvering difficult (Skerman, 1960). Biofouling of ship hulls, navigational buoys, underwater equipment, seawater piping systems, industrial or municipal intakes, oil rigs and allied structures are often reported (Fischer et al., 1981; Haderlie, 1981).

The list of affected structures has expanded in the past few decades from the increase use of the marine environment, including offshore platforms, moored oceanographic instruments and other facilities associated with aquaculture operation (Vessey & Williams, 1994; Richards & Vadua, 1980; 1981; Wilkine, 1981; Madin et al., 2009; 2010). It is estimated that the marine industry incurs an annual expenditure of 10 billion sterling pounds to combat the situations arising from biofouling worldwide (Abdul Aziz et al., 2001). Among the major problems caused by marine biofouling are increased drag and frictional resistance on ships thereby increasing 10% higher fuel consumption. Biofouling also brings severe physical stress on the structures by increasing weight and area in the current flow that can lead to breakage and failure of the system (Adkins et al., 1996; Kerr, 1998).

Biofilm, the early stage of fouling formation is also well known as biodeterioration agent in various equipment or artificial structures including facilities associated with aquaculture operations. In various industries, the formation of biofilms and slimes within pipe works, cooling systems, heat exchangers and filters can cause

problems due to resistance in pipes or decrease in heat exchange capabilities, which result in decreased production rates and increased costs (Morton & Gaylarde, 2001). Biodeterioration or biocorrosion is due to any undesirable change in the properties of a material caused by activities of organisms, particularly microorganisms. This chemical assimilatory process occurs when a material is degraded causing corrosion, pigmentation or the release of toxic metabolites compounds (Morton & Gaylarde, 2001). The biofilm also limits the use of all kinds of marine sensors (Kerr, 1998).

Many marine organisms including the cultivated organisms such as seaweeds and shellfish encounter the constant problem of being colonized and over-grown by biofouling organisms. Sessile plants and animals are generally exposed to biofouling and consequent loss of species and community assemblages (cited in Abdul Azis et al., 2001). Epibiosis or the fouling by various epiphytes and epizoans are detrimental to aquatic organisms. In gastropods, epibionts are known to promote dislodgment, reduced growth rates and fecundity as well as shell destruction leading to direct mortality (Ishac & Bishai, 1968; Lauckner, 1980; Wahl, 1989, 1996; Kumar & Ayyakkannu, 1991). Many species that are especially prone to be fouled have developed antifouling mechanisms comprising behavioural, mechanical and chemical defenses such as metabolites that are toxic to fouling (Bottjer, 1981; Wahl, 1989; Gerhart et al., 1988).

Bioinvasion or introduction of new species to a local ecosystem is another serious ecological impacts caused by biofouling. Fouling organisms on ship hulls are the main agents to spread new species. The introduction of new species has serious consequences to native biota, fisheries industries and coastal ecosystems (Anil et al., 2002). Biofouling organims have a wide tolerance range to different ecological parameters and their ability to survive in new environment contributes to their wide range of distribution.

1.1.2.2. Positive Impacts of Biofouling

The positive impacts of biofouling to human activities organisms however could not be generalized because this only happens in specific situation and localized area. The most well known is the use of certain biofouling organisms to biofilter a polluted area, for example, aquaculture farms. The release of high concentration of nutrient discharge and waste products into a polluted area will contribute to eutrophication and the use of filter feeding mechanism of several biofouling species can reduce the amount of nutrient concentration. The use of filter feeding fouling organisms can minimize the impacts of pollution in aquaculture farms (Coasta-Pierce & Bridger, 2002). Macrofouling such as by barnacles, mussels and oysters are currently being used in polyculture to reduce nutrient concentration and other particulates especially in the farm vicinity.

The formation of fouling community such as modified reef has been reported to attract other organisms such as fish into the community and thus important to fisheries. The artificial reefs with their fouling communities are called fish aggregating device (FADs) (Costa-Pierce & Bridger, 2002). Coastal structures such as moorings and jetties can provide settlement structures for various biofouling organisms. Petroleum platforms in offshore water serve as habitats for reef fish assemblages (Hastings et al., 1976; Gallaway & Lewbel, 1982; Seaman et al., 1989; Dokken, 1993). The man-made structures provide hard substrates for biofouling which increases the supply of prey, shelter and settlement habitat for several important species.

1.1.3. Control of Biofouling Organisms

Biofouling is considered a key impediment to the use of various equipment in the aquatic environment. According to Adkins et al. (1996), treatments to prevent the accumulation of marine life on ships' hulls have been used since historical times. There are various ways of controlling biofouling depending on the type of biofouling and the

surface structures they occupied. Some methods are used to prevent early settlement, destroy the developed fouling community regularly to avoid excessive settlement, while others prevent the spread of fouling. The methods of controlling biofouling include the use of antifouling chemical biocides and biological strategies. Both methods have limitation on their deployment and depend on the type of structure, location and the purpose of their requirement.

Historically, biofouling control using chemicals has been achieved by exploiting the toxicity of metal, organometals and other biocides to marine invertebrates and incorporating them in antifouling coatings (Anonymous, 2003). The most common biocides used in modern antifouling coatings on ships hulls and other hard surface structures are tributyl organotin (TBT) and cuprous oxide (Bleile & Rodgers, 1989). Another chemical used to control biofouling is chlorination. It is commonly used to control zebra mussel fouling on power plants and is effective for both adult zebra mussels and settling postveliger larvae (Jenner, 1985; Klerks & Fraleigh, 1991; Fraleigh et al., 1993; Van Benschoten et al., 1993; Menis-Croxall & de Bruyn, 1997; Bidwell et al., 1999). Although the chemical coatings are highly effective in controlling the fouling, their applications have been prohibited due to their toxicity and growing environmental concern (Champ, 2000; Madin et al., 2009)

The use of lethal limit of certain organisms tolerant to environmental parameter has also been used to prevent biofouling development or at least reduce the biofouling population. The heat treatment for example is as an effective fouling control measure (Masilamonia et al., 2002). The upper lethal limit of sea water temperature for most marine organisms is around 37 ^{o}C and further increase may reduce or kill the organisms (Iwanyzki & McCauley, 1993; Jenner et al., 1998). This method is preferably useful in tropical seas since the organisms are living in temperatures closer to their upper lethal

limit but it important to identify the temperature tolerance of species and their physiological responses (Jenner et al., 1998; Masilamonia et al., 2002).

1.2. Overview of Aquaculture

Aquaculture is well known as the fastest growing industry world-wide. Among the reasons for this include the depletion of wild fish stocks, increasing demand for seafood or freshwater aquatic species as the main source of animal protein and the shortfall of wild fisheries landing around the world. Aquaculture in general is equivalent to underwater agriculture activities to produce aquatic animals and plants under controlled conditions using marine or freshwater resources (Stickney, 1994; FAO, 1995). Marine aquaculture or mariculture refers to the production of marine organisms which is less inclusive than aquaculture which relates to both marine and freshwater environment (Stickney, 1994). The main purpose of aquaculture is to produce fish food for human consumption including various species of finfish, shellfish and seaweed, but with the rise in concern for species preservation especially endangered wild species, the purpose of aquaculture has become more diverse. Some other organisms which are of no interest for aquaculture in the past, has now been identified for use in various drug productions and this requires potentially large aquaculture industries.

The history of aquaculture is thought to begin in China since 4,000 years ago, but in many countries, aquaculture has only been practiced since one or two centuries ago while the serious involvement of scientists to conduct aquaculture research only started in 1960s. Since then aquaculture production has risen tremendously (Stickney, 1994). The improvement in production over the past few decades is attributed to good development of management techniques including water quality, disease control, feed nutrition and improvement of breed stock with application of scientific technology. Several species that could not be reared in the past are now being produced with the

advancement of aquaculture technology.

Many Asian countries with subtropical and tropical climate are the main contributors of aquaculture production because of suitable growing conditions year round. Almost 90% of the world's aquaculture production came from the Asian region (Anonymous, 1995; FAO, 1999; Crespi, 2005; FISHSTATS Plus, 2006). Low capital investment in many Asian countries is another factor thought to contribute to higher aquaculture production. The low cost of land and labor, abundant and undeveloped coastline and less stringent environmental regulations have contributed to the establishment of large aquaculture industries. China remains the largest producer, accounting for 60.4 – 67.3% of total world production (Subasinghe et al., 1996; FAO, 1999; FAO, 2007). Thailand, Indonesia, India and other Asian countries were amongst the main seafood producers in the world.

The global aquaculture production in 1997 totaled 28.8 million tons of finfish, crustaceans, and mollucs worthed US$45.5 billion and 7.2 million tons of seaweed worthed US$4.9 billion. However, the production needs to be expanded to 62 million tons by the year 2035 to maintain global consumption (FAO, 1999; New, 2000; FAO, 2007).

1.2.1. Types of Aquaculture

Many methods of aquaculture are used in fresh, brackish and marine water environments. Natural ecosystems such as lakes, water reservoirs, mangrove estuary and coastal zone are commonly used to practice aquaculture. The earliest method of aquaculture is the rearing of fish in ponds which is still practiced in many countries. Several other methods have since been developed to increase production such as net-cages, pen cultures and raceways that can be classified into different levels as either extensive, semi-intensive or intensive system.

Net-cages and pen culture viewed as aquaculture systems for the new millennium and are becoming very popular among culturists and their production of fish in both marine and fresh water has contributed significantly to fish supply (Chua & Tech, 2002). Both the net-cage and pen cultures are types of enclosure culture holding captive fish within enclosed spaces and maintaining free exchange of water. The net-cage system is totally enclosed on all sides by mesh or netting, while the pen culture is an enclosure with its bottom formed by the lake or sea bottom (Beveridge, 1984).

The use of net-cage is a more efficient and economic way of raising fish compared to the use of pen culture (Chua & Tech, 2002). The earliest record of cage culture activities was in Southeast Asia in the 1800s. Wood or bamboo was used to construct a cage for snakehead (*Channa* sp.), catfish (*Pangasius* sp.) and gobies (*Oxycleotris* spp.) and fed with trash-fish and food scraps in freshwater lakes and rivers in Cambodia (Coche, 1976; Pantulu, 1976 cited in Chua & Tech, 2002; Beveridge, 1987). Net-cage aquaculture has several advantages because it can be deployed in many types of water bodies including lakes, reservoirs, ponds, strip pits, streams and rivers. Furthermore, it requires comparatively low capital investment and uses simple technology (Beveridge, 1984; 1996).

There are various types of net-cage culture being developed, including fixed net-cages, floating net-cages, submersible net-cages, rotating and non-rotating floating net-cages. The fixed net-cage types consist of a net bag supported by posts driven into the bottom of a lake or river. It is a stationary cage which is fastened by fixed bamboo or wooden poles at its corners. The floating net-cage consists of a floating unit from which a single cage or a series of net-cages is suspended. It consists of floats, framework and net-cage (Madin et al., 2009; 2010). The submersible net-cage is a type of design which has no collar, and the bag rests on a frame to maintain its shape. Its position with reference to the water column can be adjusted by means of buoys. The rotating and non-

rotating net-cage is designed primarily to reduce biofouling impacts. The net-cage rotates from a central axis attached to a solid floating framework (Christensen, 1995) while the non-rotating type is designed with a narrow or wide collar.

The net-cage culture system can be classified as extensive, semi intensive and intensive based on type of species cultured, the feed input, labor and other facilities needed to manage the farms. In extensive culture, fish rely on the natural productivity of water such as plankton and seston carried in the drift. This system requires no external feeding and is usually used to culture omnivorous or herbivorous fish. However its use is limited because there are few commercial species that feed on plankton. Semi-intensive culture involves the use of low protein (<10%) feedstuff that is usually compounded from locally available plant or agriculture by-products to supplement the production of natural food. In intensive culture, cultured fish rely almost exclusively on an external supply of higher protein (>20%), nutritionally-complete food and other mineral required by fish which usually based on fishmeal (Coche, 1982; Beveridge, 1987).

The use of net-cage culture system is commonly practiced in various countries but its rapid growth is a relatively recent phenomenon (Beveridge, 1996; Beveridge & Little, 2002). According to Myrseth (2000), the net-cage culture system is an important and most productive system of rearing fish; it has been changing rapidly following the industry's best interests from technological, market and regulatory perspectives. Its tremendous growth is attributed to several factors such as high market value of marine fishes, advancement in net-cage technology under various oceanographic conditions, technical support and good quality input of feed, fry, etc. With the advancement in net-cage technology, the number of commercially-important fish species cultured in net-cage aquaculture has increased tremendously. According to Islam (2005), there are more than 50 commercially-important fish species cultured in net-cage aquaculture,

while Chua & Tech (2002) indicate that tens of finfish species have been cultivated in various designs and sizes of net-cage systems all over the world. For example, tilapia and carps are predominantly farmed in Asia while salmonids are commonly farmed in Europe and America.

The use of net-cage aquaculture system for large scale production of commercial finfish is an expanding industry in many countries (Ackefors & Olburs, 1996; Makinen, 1991; FAO, 2001; Subasinghe, 2004). In the early 1950s, world production of net-cage farming was about 100 ktons but production increased to about 1500 ktons in 2002 and is expected to reach 2000 ktons by 2010 (Subasinghe, 2004). The number of net-cages aquaculture and production are still increasing in several Asian countries including Malaysia (Liao & Lin, 2000; Takashima & Arimoto, 2000; Madin et al., 2010). A similar trend is also observed in Europe (Enell, 1995; Piedrahita, 2003).

The net-cage culture industry is a relatively recent development in Malaysia. Large-scale farming in marine waters started in the 1980s and has since expanded rapidly (Chong, 1998; Shariff & Gopinath, 2000; Madin et al., 2010). Its growth is nearly six times faster than the growth of shrimp pond cultivation as it becomes more popular among the fish farmers (Chong, 1998). It is now the fastest growing sector in the aquaculture industry, with 82,800 net-cages (1,314,151.15 m^2) which produced 15,122.82 tonnes in 2007 (Anonymous, 2007; Madin et al., 2010).

Among the popular fish cultured in floating net-cages in Malaysia are the giant seabass (*Lates calcarifer*), golden snapper (*Lutjanus johnii*) and red snapper (*Lutjanus argentimaculatus*) (Shariff & Gopinath, 2000; Madin et al., 2009; 2010). Malaysia is among the largest producers of seabass cultured in floating net-cages in Southeast Asia (Rimmer & Russel, 1998). The use of cheap fish feed such as ground trash-fish for cultured marine fish in floating net-cage is widely practiced in Malaysia and other

Southeast Asian countries. Trash-fish is available from the by-catch of fishermen and relatively cheaper compared to largely imported commercial pellet feed (Madin et al., 2009; 2010).

1.3. Biofouling in Aquaculture and Impacts

The biofouling problem is more serious in open system aquaculture where submerged in-situ facilities such as net-cages and culture pens are used. According to Hodson et al. (1995; 1997) biofouling on net-cages netting is a serious problem to mariculture management worldwide. The biofouling in aquaculture is a constant operational concern whether in the marine or freshwater environment; in tropical waters, it is a serious problem particularly in net-cage culture operation (Chua & Tech, 2002; Madin et al., 2009; 2010).

Biofouling of net-cages will significantly reduce the size of net mesh, impedes water flow through the net (Milne, 1976; Faure, 1986; Huse et al., 1990; Madin et al., 2009; 2010) and therefore the supply of dissolved oxygen to the cultured fish resulting in serious asphyxiation problem (Inoue, 1972; Ojeda & Strawn, 1980; Aarsnes et al., 1990; Loland, 1993). Net biofouling increases the accumulation of waste products including uneaten food and other metabolite waste and thus further exacerbates water quality problem (Inoue, 1972; Porter, 1981; Blair et al., 1982; Huse et al., 1990; Aarsnes et al., 1990; Madin et al., 2009; 2010). Deteriorated water quality delays cultured fish growth especially of fingerlings (Moring & Moring, 1975). The fouled net will be heavier, thereby increasing the drag force and structural fatigue (Milne, 1970; Huguenin & Ansuini, 1978). Increased drag due to wind conditions, waves, and strong water flow will increase the possibility of net rupture or net-cage collapse especially if the water flow is at its maximum (Beveridge, 1996; Phillippi et al., 2001; Swift et al., 2006; Madin et al., 2009; 2010).

Biofouling in aquaculture requires frequent and expensive cleaning of nets, which increase net damage, loss of stocked fish during net changes, and disturbance of feeding regimes causing lower growth rates of cultured fish (Huguenin & Ansuini, 1978; Hodson et al., 1995; 1997; 2000; Madin et al., 2009; 2010). The salmon industry in Australia for instance requires cleaning every 5 – 8 days during the summer seasons (Hodson & Burke, 1994), which involves up to 20 – 38% of the total aquaculture labour requirement (Huguenin & Ansuini, 1978). Net changing and cleaning incurs a major cost as it is necessary to purchase a large number of nets, and to employ dedicated net-changing and cleaning personnel (Hodson et al., 1997). The wooden structures of aquaculture facilities are also affected by fouling (boring) organisms such as *Martesia* sp. that cause serious damage of the net-cage units (Milne, 1970; Cheah & Chua, 1979).

Net-cage biofouling has also been speculated to be a potential reservoir of disease-causing microorganisms such as *Neoparamoeba pemaquidensis* responsible for the amoebae gill disease (AGD) (Alexander, 1991; Kent, 1992; Clark & Nowak, 1999; Nowak, 2001; Tan, 2002). According to Cundell & Mitchell (1977) and Alexander (1991), biofouling of net-cages increases the incidence of amoebae gill disease by providing suitable surfaces for amoebal attachment and growth while dissolved and particulate matter provide the food source. Studies of microfouling communities on salmon net-cage netting have revealed a high number of protista (Hodson & Burke, 1994). However, type of microorganisms varied depending on the substrate and environmental conditions (Corpe, 1976; Dempsey, 1981a; 1981b; Hodson & Burke, 1994).

Several factors are thought to influence biofouling development on net-cages. The rate of biofouling depends on several factors including net mesh size, productivity of the farms, general climate and season (Moring & Moring, 1975; Milne, 1976; Madin et al.,

2009; 2010). According to Dubost et al. (1996), fouling settlement depends on submersion time, net surface and the species present in the water. For a given biofouling organism, settlement on and eventual clogging of the net depend on mesh size (Cheah & Chua, 1979), as well as the type and physical characteristics of the netting material (Dubost et al., 1996). The nutrient enrichment derived from waste products of feed and fecal material in the fish farm is also thought to influence biofouling rates (Madin et al., 2009; 2010). Organic pollution is another important factor in the development of biofouling. However these factors also interact with physical factors such as salinity, temperature, turbidity and water motion in a way that makes biofouling development a complicated succession of sessile and associated vagile organisms (Madin et al., 2009)

In aquaculture, mechanical method of cleaning the fouled netting material is still the most efficient and cheapest way to remove biofouling organisms. Fouling is typically managed by regular net-cage changes and cleaning with high-pressure water (Hardy, 1991; Laing & Spencer, 1997). The method used is simply replacing the biofouling infested net-cages and allow it to dry after which a high-pressure water pump is then used to purge the biofouling and other debris materials entrapped on the net mesh (Madin et al., 2009; 2010). For the small scale farms, a rotating cylindrical net-cage can delay biofouling development (Caillouet, 1972).

The use of chemical method to control biofouling in aquaculture has stopped in some countries due to concern of environmental effects and consumer preferences that may jeopardize the market image (Hodson et al., 1997; Champ, 2000; Braithwaite et al., 2007; Madin et al., 2009). Various attempts have been made to exploit natural antifouling chemicals from marine plants and animals for use in aquaculture industries (Armstrong et al., 1999; Harder & Qian, 2000). However, these methods are still the expensive solutions. Biological method to reduce fouling such as the use of competing

filter-feeding macrofauna and other invertebrates have been introduced in aquaculture but has yet to be widely practiced and their effectiveness is still in doubt.

1.4. Significance of Study

The fisheries sector in Malaysia plays an important role in providing fish as a source of food and protein. It contributes 1.6% to the National Gross Domestic Product (GDP) and directly provides employment to 81,994 fisherman and 21,774 fish culturist (Anonymous, 2000a). In the year of 2000, the total of fisheries sector accounted for 1,453,590 tonnes valued RM 5.37 billion. Of this production, total aquaculture produced 167,894 tonnes or 11.55% of the total production. The production from brackish water aquaculture was 117,206 tonnes or 69.8% of the total aquaculture production, with the market value of about RM 607.75 million (Anonymous, 2001).

As fish production from the wild reaches its maximum or decline, aquaculture is expected to become a major contributor to the country's economy. According to the long term National Agriculture Policy (NAP), aquaculture production has been projected to reach 600,000 tonnes through the development of some 35,000 ha of surface area of land and water where the projected figure for brackish water culture alone is 400,000 tonnes and are expected to be produced from 20,000 ha of land or water area (Anonymous, 1993; Anonymous, 1999b; Anonymous, 2000b; Madin et al., 2010). Of this production, 120,000 tonnes of marine fish are expected to be produced from largely fish net-cages in marine waters which is deemed to be the most productive system in term of production per unit surface area (Chong, 1998).

To achieve the long term goal of NAP, the Fisheries Department has formulated the Aquaculture Development Action Plan (ADAP) that is responsible to the expansion of various aquaculture facilities such as net-cage, pond, raceway and others (Anonymous,

1993; Anonymous, 1999b; Anonymous, 2000b). The aquaculture development will focus on conservation and utilization of fisheries resources on a sustainable basis. It will be adequately supported by modern fisheries' infrastructure, processing, marketing network, comprehensive human resource development (HRD) and R&D programmes such as production of fish fry, fish feed as well as fish farm management including fish diseases, water quality and biofouling associated problem.

The intensification of aquaculture production will however require the use of larger feed inputs including formulated feed and trash-fish feed. In particular, the demand for high value species such as groupers, snappers and giant sea perch will require high inputs of fish protein in their feed. The amount of formulated feed required by the aquaculture industry by the year 2010 has been estimated at 690,000 tonnes, including 465,000 tonnes for fish and 225,000 tonnes for marine shrimp; only 100,000 tonnes can be produced locally and the balance will be imported especially in the form of specially formulated feed (Che Musa & Nuruddin, 2005).

In Malaysia, biofouling development in fish culture such as in floating net-cages (Plate 1.1) is a major problem for the management and thus productivity of fish farms. The fouled net has to be removed (Plate 1.2) and replaced with new net-cages. In small fish farms, the method adopted is to first dry the removed net (Plate 1.3a & 1.3b) for several days under the hot sun and then breaking up the dried shells and crusts using a hammer. The net is then cleaned of its burden using a high pressure water pump which incurs high power and labour cost (Plate 1.3c). This method is not only labour-intensive but also damages the net over the long term. Net-cages experience biofouling problem within 3 – 4 weeks of culture, and failure to conduct net changes (after heavy fouling) often results in water quality problem due to impeded water flow (Plate 1.4) and net strain due to the fouling weight. The consequence could be fish asphyxiation and lost of

(a)

(b)

Plate 1.1. (a) Floating net-cages with fouled fish netting showing (b) biofouling organisms that almost entirely covered the net-cage apertures.

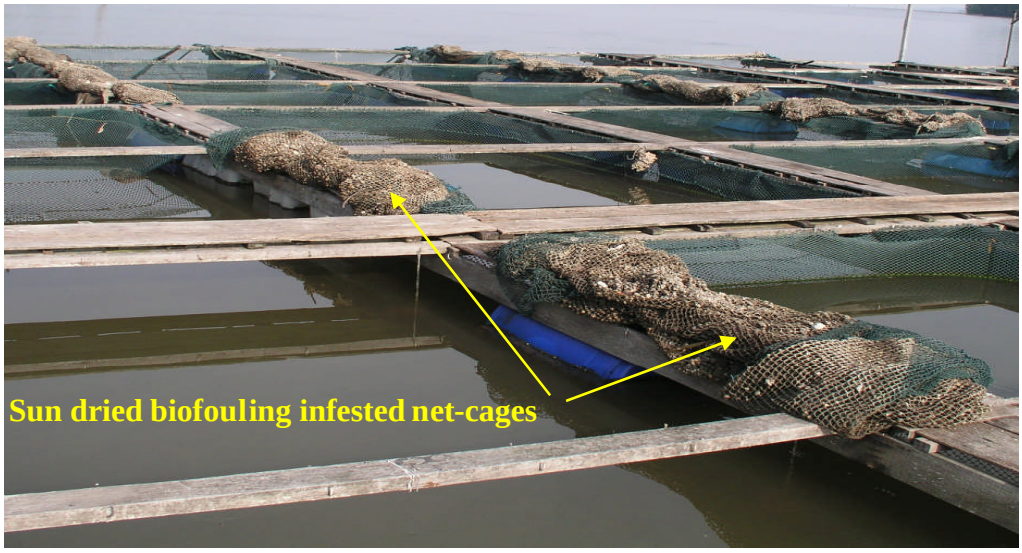
(a)

(b)

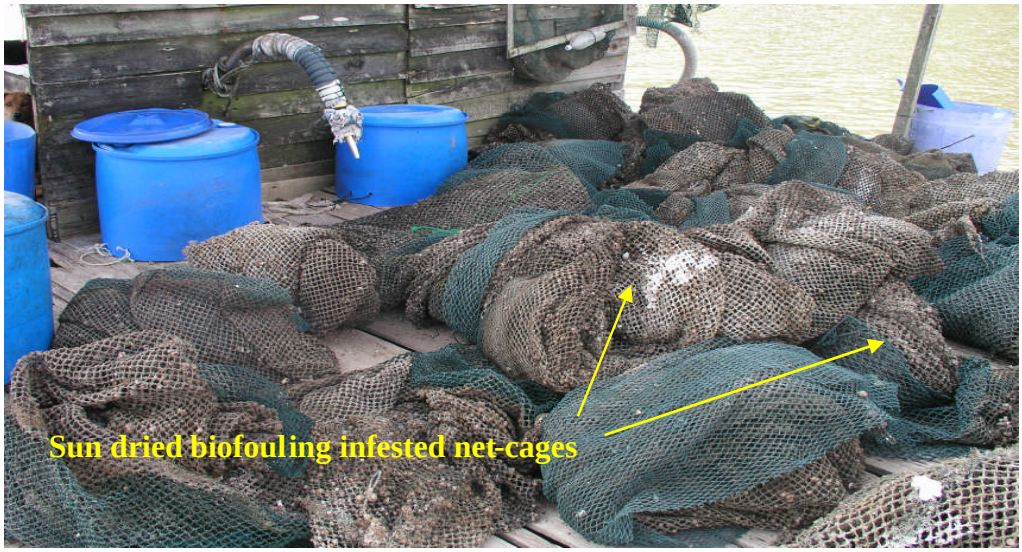
(c)

Plate 1.2. (a, b & c) Biofouling infested net-cages, removed and replaced with new and unfouled net-cages. At least two personnel required to carry out the task.

(a)



(b)



(c)

Plate 1.3. (a) Removed biofouling infested net-cages dried under the hot sun, (b) net-cages gathered to breakup hard fouling, and (c) their biofouling removed using high pressure water pump.

(i)

(ii)

Plate 1.4. (i & ii) Mortality of cultured fish inside the floating fish cages due to depletion of dissolved oxygen concentration exacerbated by biofouling organisms which impeded water flow through the net-cages.

cultured fish in the event of strong tidal flow. The operation of changing cage nets often injures and stresses the cultured fish which may result in mortality (see also Madin et al., 2009; 2010).

As the numbers of marine cage-net farms are expected to increase in line with the country's long-term Aquaculture Development Action Plan (ADAP) to expand the sector by an annual rate of 20%, net-cage units are expected to crowd in limited water space such as in tidal estuaries and protected coastal waters. Net biofouling is thus expected to be an important problem, not only as an operational cost but also as a production liability if reduced fish growth and mortality occur. Furthermore, the expansion of net-cage culture will require the use of larger fish feed input particularly poor quality feed such as ground trash-fish which is also expected to contribute to the high biofouling rates as well as deteriorations of water quality (Madin et al., 2009; 2010). Research on biofouling associated with aquaculture is almost non-existent in Malaysia. More research particularly on biofouling control and management is required to help reduce costs due to the negative impacts of biofouling development. The present study is thus carried out to contribute to the poor knowledge of tropical biofouling in floating net-cage aquaculture.

1.5. Scope and Overall Objective of Study

The main focus of this study was on the development of macrofouling organisms with a size range of 125 μm and above. Two components of macrofouling organisms were studied, sessile organisms and non-sessile associates. For the purpose of this study, the non-sessile associates are considered as foulers on the nets, since they occupied space within the sessile community, or are tube dwellers or burrowing into accumulated sediment, and are able to form colonies on the fish cage nettings. The overall objective of the present study was to elucidate the short-term colonization dynamics of

macrobiofouling organisms on fish cage nettings and the factors that influence biofouling development in the tropical marine environment.

In order to achieve the overall objective of this study, the following studies were conducted:

1) The community structure, short-term colonization dynamics and biomass of macrofouling assemblages on net-cages, in relation to fish rearing, type of fish feed and season (Chapter 3).
2) Effects of fish rearing, fish feed, water flow and net-cage position in the fish farm on biomass of net biofouling organisms (Chapter 4).
3) The effect of salinity on macrofouling community structure on nets (Chapter 5).
4) Biofouling development in relation to nutrient and chlorophyll-*a* concentration of culture water (Chapter 6).

CHAPTER 2

STUDY AREA AND GENERAL METHODOLOGY

2.1. Study Area

The study was carried out in Matang Mangrove Forest Reserve (MMFR) (4º 15' N 100º 2'E to 5º 1'N 100º 45') situated in the state of Perak, Peninsular Malaysia (Figure 2.1). It is the largest mangrove forest in Peninsular Malaysia and is well known as the best managed mangrove forest in the world. It occupies a total area of 40,711 ha and composed mainly of silvicultured *Rhizophora apiculata* mangroves (Gan, 1995). Deltaic islands of MMFR are separated by numerous rivers and waterways which are suitable for fish farming and other fisheries activities. The MMFR is setup and managed on a sustainable management basis since 1902 by the Perak State Forestry Department.

The MMFR has an equatorial type of climate with relatively humid and higher temperature throughout the year. The temperature ranges from 21 Cº – 34 Cº and the relative percentage of humidity was 82 – 86% (Gan, 1995). Rainfall is experienced throughout the year, but there are two seasonal peaks coinciding with the onset of the southwest and northeast monsoons in May and November respectively. The total rainfall was 231 – 390 cm a year (Lee et al., 1993). The MMFR receives numerous freshwater inputs through various rivers and waterways (see Figure 2.1). Water is well mixed and the tides are semi-diurnal with a Mean High Water Spring (MHWS) of 2.65 m (Sasekumar et al., 1994; Chong, 1999; Tanaka & Choo, 2000). The mean tidal range is 3.3 m.

The numerous river and waterways provide the nursery and feeding areas for various commercially-valuable marine organisms (Chong, 2006; 2007; Chew et al., 2007; Then, 2008). The floating net-cage culturing fish and thriving cockle culture are among the important aquaculture activities that develop rapidly in this area. Net-cage culture for

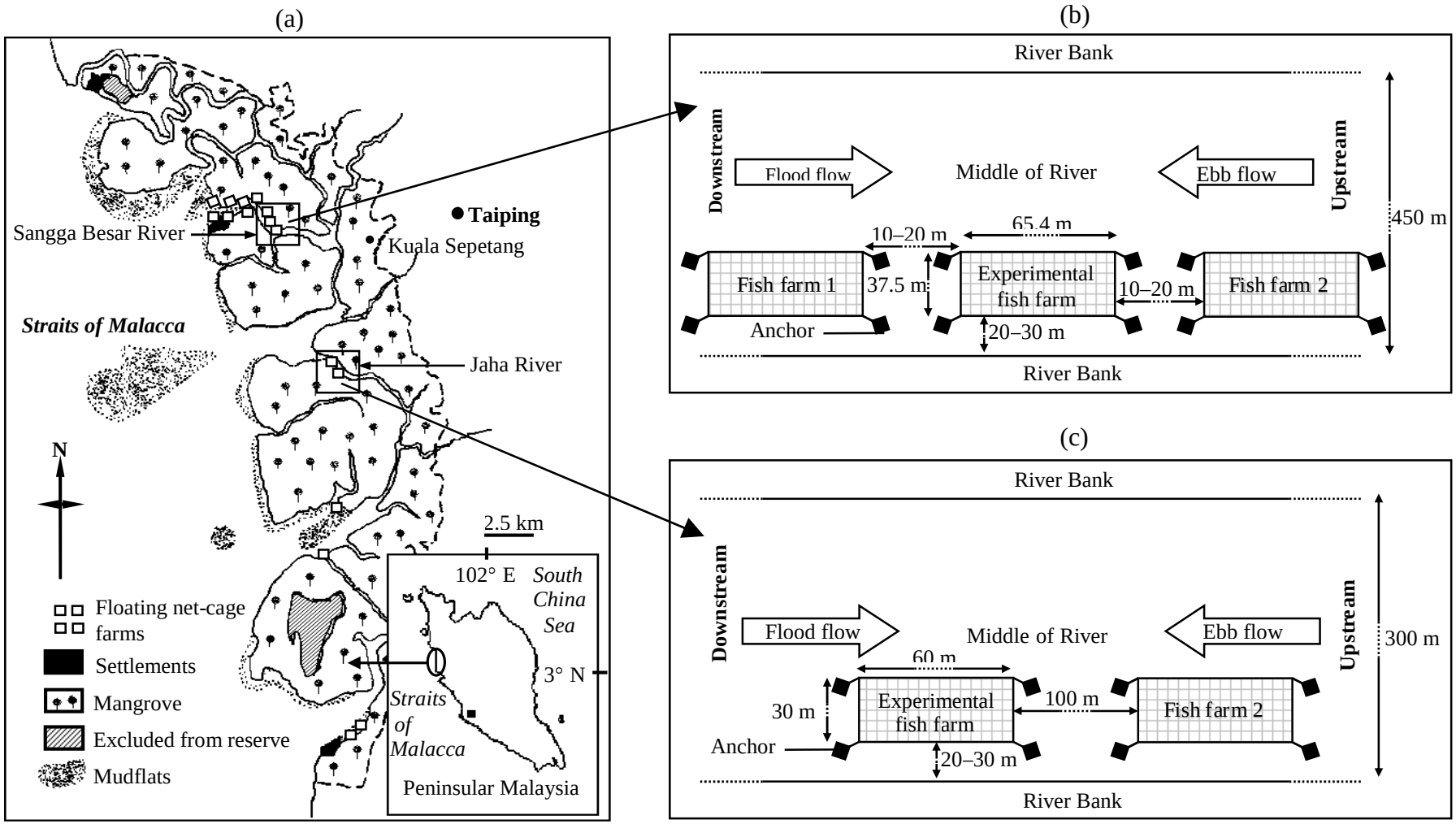


Figure 2.1. (a) Location of study site at Sangga Besar and Jaha River estuary in the Matang Mangrove Forest Reserve (MMFR), Perak, Peninsular Malaysia. Floating net-cage farms located along the estuaries of (b) Sangga Besar River and (c) Jaha River.

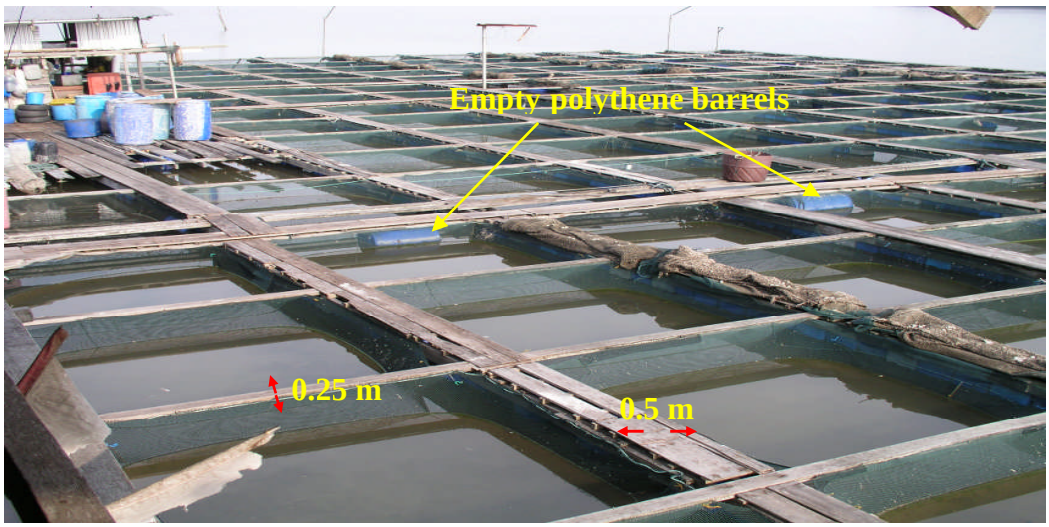
fish is increasingly becoming more important. In 1989, there were 902 net-cages located in the lower reaches of Sangga Besar River. Currently, there were approximately 4,000 cage units were present in Sangga Besar River. The culturing activities heavily occur in the Kuala Sepetang area.

Each fish farm consists of a series of interconnected floating net-cages (Plate 2.1). Among the popular species cultured are seabass (*Lates calcarifer*), golden snapper (*Lutjanus johnii*) and red snapper (*Lutjanus argentimaculatus*). The main fish feed given to the cultured fish are trash-fish, comprising mainly of young slender shad (*Ilisha elongata*), gizzard shad (*Anadontostoma chacunda*) and thyrssa anchovy (*Thryssa kammalensis*) (Plate 2.2). The frequency of trash-fish feeding is dependent on tide. During the spring tide feeding is normally once a day but during neap tide, it is twice a day. The weight of trash-fish feed given per net-cage varies from 8 – 15 kg day^{-1} for adult fish and 2 – 4 kg day^{-1} for juvenile fish. The cultured fish are normally ready to be harvested after 7 – 8 months or when the fish average size reaches 600 g. However, the harvesting season also depends on market demand.

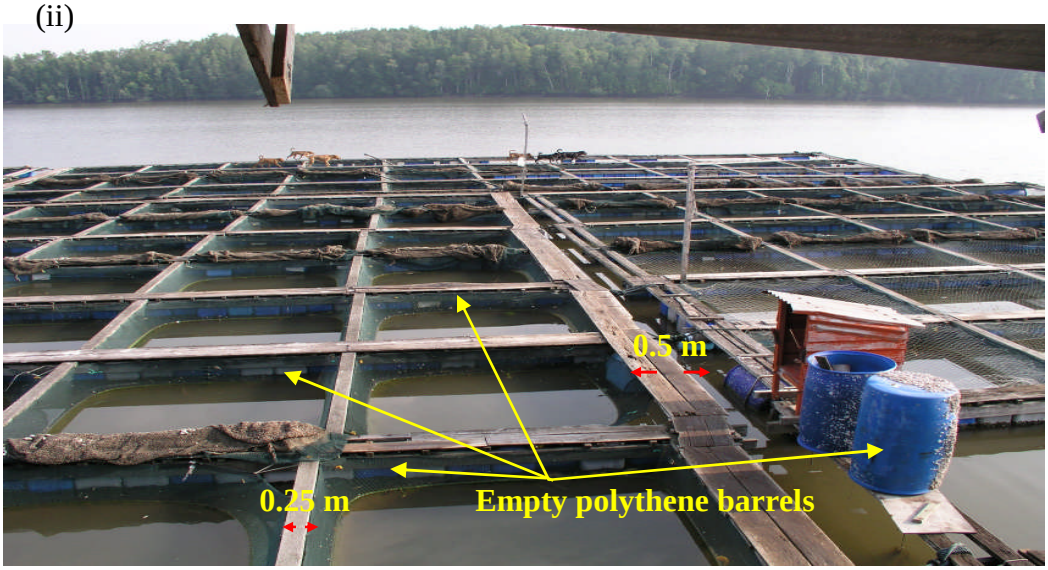
For the purpose of this study, two floating net-cages farm located at Sangga Besar River (see Figure 2.1b) and Jaha River (see Figure 2.1c) were selected. Fish net-cage farms are particularly dense (5,964 net-cages) in the estuary of Sangga Besar River (Plate 2.3a). However in the Jaha River, there were only two farms with a total of approximately 600 floating net-cages (Plate 2.3b). The Jaha River estuary is shallow averaging 3 m depth. Water is well mixed and the tides are semi-diurnal, with a tidal amplitude of 2.5 m. Among the reasons for selecting the two sites was to compare biofouling development between the high-density net-cage farms in Sangga Besar River and the low-density farms at Jaha River estuary.

The floating net-cage farms in both estuaries of the Sangga Besar River and Jaha

(i)



(ii)



(iii)

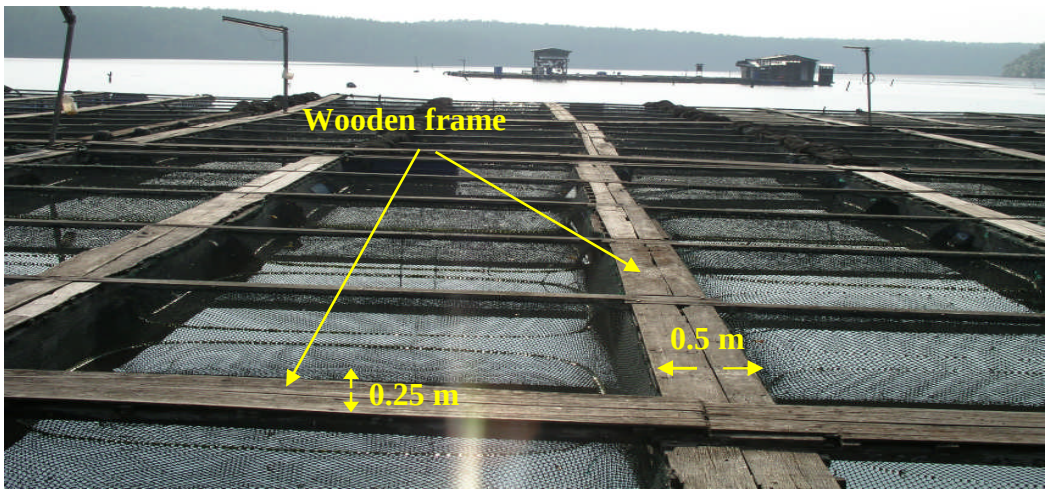


Plate 2.1. Typical type of interconnected floating net-cages in a fish farm at MMFR. The net-cages are arranged in series with a wooden frame of 0.5 m between the adjacent net-cages series in row, which also acts as a walk way in the farm. The 0.25 m gap separates each row of net-cage units. Fish farm kept afloat through the use of empty polythene barrels.

(a)

(b)

(c)

(d)

Plate 2.2. (a) Trash-fish feed comprising mainly of young slender shads, (b) unground trash-fish feed for matured fish, (c) ground trash-fish feed for fingerlings, and (d) fish feeding carried out in a fish farm during slack water.

(a)

(b)

Plate 2.3. (a) Dense fish farms in Sangga Besar River, and (b) experimental fish farm at Jaha River.

River were located approximately 20 – 30 m from the river bank and extended another 30 m to the middle of the river (see Figure 2.1b & 2.1c). The farm was permanently positioned with the use of metal and concrete anchors at both ends and kept afloat through the use of empty polythene barrels. The net-cages were arranged in series with a wooden frame of 0.5 m between adjacent net-cages row (see Plate 2.1).

2.2. Experimental Design and Layout

Four studies were conducted to investigate the causes and development of biofouling on fish cage nettings. The first study investigated the community structure, short-term colonization dynamics and biomass of macrofouling assemblages on nets in relation to fish rearing, type of fish feed input and season. The subsequent three studies were carried out to test the proposed hypotheses arising from the first study. The second subsequent study examined the effects of fish rearing, fish feed, water flow and net-cage position in the farm on the biomass of biofouling organisms. The third study examined the effects of salinity on the macrofouling community structure on nets. The fourth study examined the nutrient and chlorophyll-*a* concentrations of culture water inside the net-cages to determine their possible relationship with biofouling development. During these experiments, water parameters were also measured to study their possible affects on biofouling development. The general experiment layouts of each study are given as follows:

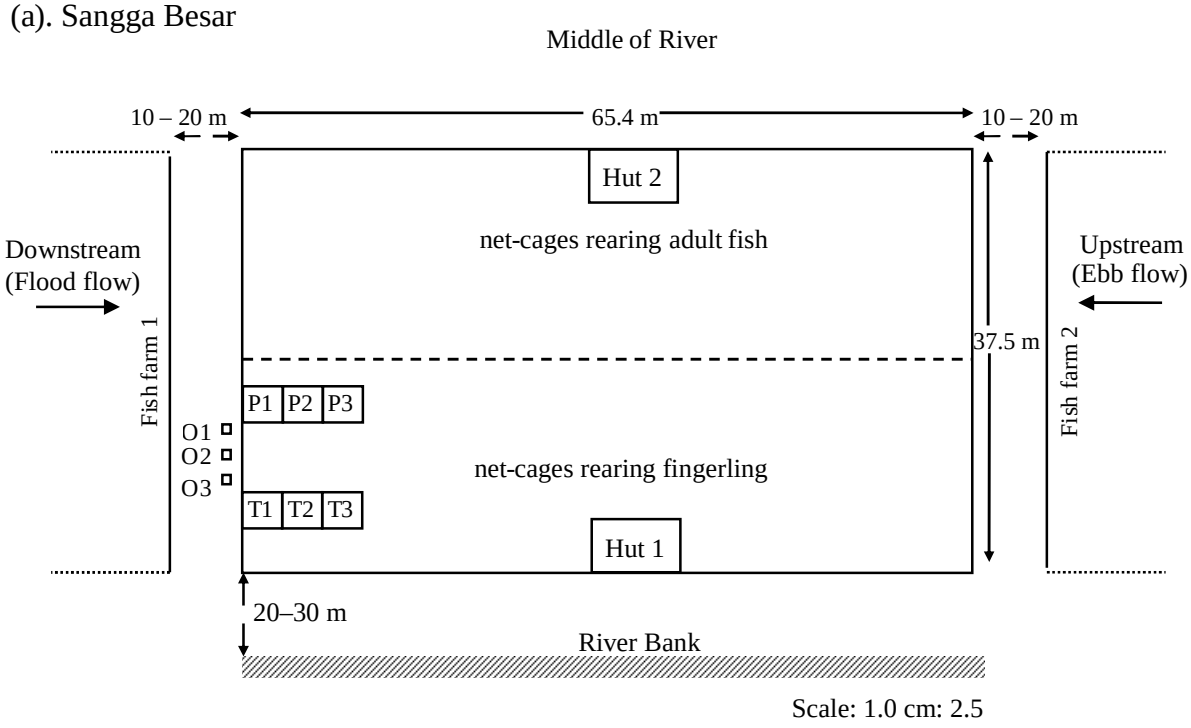
2.2.1. Study on Community Structure, Short-Term Colonization Dynamics and Biomass of Macrofouling Assemblages on Cage Nets (Chapter 3, page 68 – 165)

This study examined the species composition, abundance, colonization dynamics, including the depth distribution of sessile organisms and total wet biomass of macrofouling assemblages in relation to fish rearing, type of fish feed input and season.

2.2.1.1. Experimental Method and Sampling

The experiments were conducted at two fish farms in the estuaries of Sangga Besar River (Figure 2.2a) and Jaha River (Figure 2.2b) during the dry season (July –August 2000) and at one fish farm in Jaha River during the wet season (November 2000 – January 2001). During the wet season, the experiment at Sangga Besar had to be terminated due to disease outbreak which had caused significant mortality of the cultivated fish. A total of six, clean and unfouled floating fish net-cages with nettings of 1.6 cm mesh size (or 2.3 cm stretched mesh size) suitable for stocking fingerlings were used for each experiment at both locations and seasons. The experimental net-cages measured 2.5 m x 2.5 m in surface area, with a net depth of 1.5 m and 2 m for Jaha and Sangga Besar farm respectively. The net-cages were set up in triplicates to receive either ground trash-fish (T1, T2 and T3) or pellet feed (P1, P2 and P3). The use of ground trash-fish is the common practice of fish feeding at MMFR. To ensure that all experimental net-cages were exposed to the same or near similar current regime, experimental net-cages were positioned at the downstream end of the farm (see Figure 2.2a & 2.2b). This selection was also to minimize cross-contamination during fish feeding carried out at low slack tide, although feed given were completely consumed by fish within the first 15 minutes. The experimental net-cages were set up within the fingerlings' rearing area of the fish culture farm where high macrofouling rates were expected to occur due to the use of small mesh cage nets. The limited space for rearing fingerlings only allowed the triplicates to be arranged linearly in triplets (i.e. along main axis of river) at one half of the small farm. Nevertheless, each member of a treatment triplet (e.g. T1) was assumed to be exposed to similar physical conditions as their counterpart (P1) on the other treatment triplet. Preliminary current measurements inside similarly arranged net-cages had shown that the flow rates were not significantly different among members of a triplet, except at the start of the experiment when the nets were clean but even then the

(a). Sangga Besar



(b). Jaha

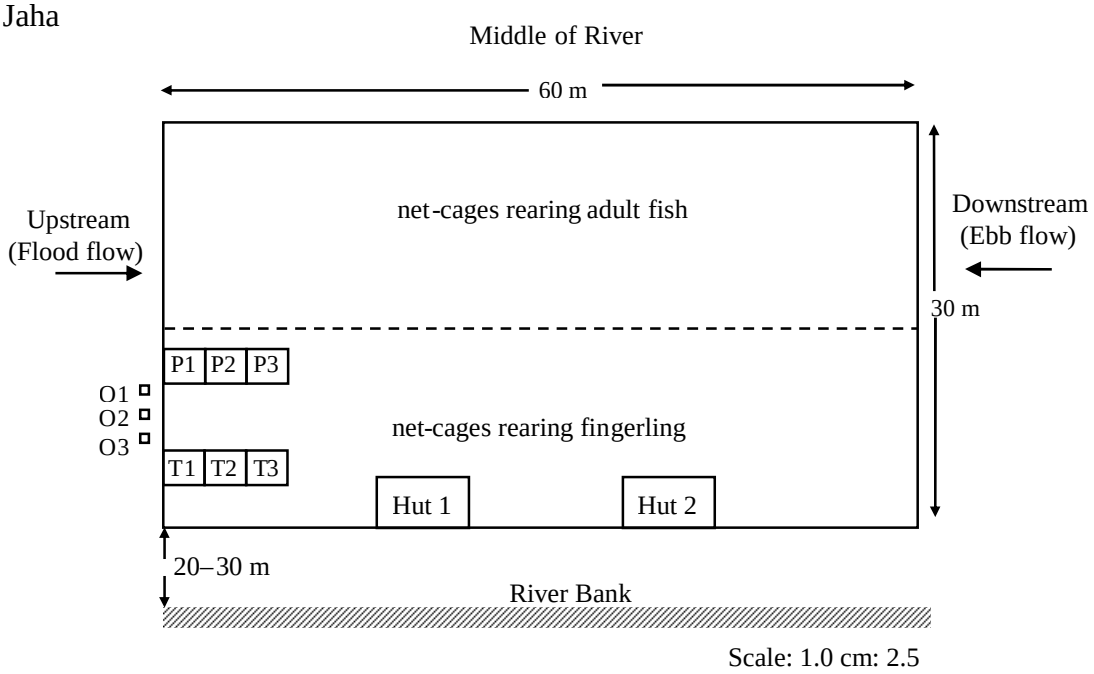


Figure 2.2. Sketch diagrams of (a) fish farm at Sangga Besar River estuary measuring 65.4 x 37.5 m, containing 272 net-cage units which is positioned at 10 – 20 m distance from neighbouring fish farm 1 & 2, and (b) fish farm at Jaha River estuary measuring 60 m x 30 m, containing 258 net-cage units which is positioned approximately 100 m distance from neighbouring fish farm. T1, T2 and T3: net-cages given trash-fish feed; P1, P2, and P3; net-cages given pellet feed; O1, O2, and O3: without net-cages (i.e. outside the net-cage farm, without fish and feed). See Figure 2.1. for details.

current velocity became rather homogenous across the farm as biofouling progressed.

Each experimental net-cage were initially stocked with 200 giant sea perch (*Lates calcarifer*) fingerlings (mean wet weight of 27 ± 4.5 g) and daily feed rate was 3 – 4% of the total biomass of stocked fish. The home-made pellets were rod shaped and made from poultry offal meal at a mean size of 2 mm diameter. Percentage content of crude protein, crude lipid and ash based on dry weight were 42.5%, 12.6% and 13.1% respectively. Moisture content was 5.4%. Trash-fish were ground to a sticky pulp containing particulates of very variable sizes. Its percentage content of crude protein, crude lipid and ash based on dry weight were 75.4%, 5.9% and 18.6% respectively. Moisture content was 75%.

Multifilament nylon net panels each of 0.2 m x 2.0 m dimensions with 1.6 cm mesh size (or 2.3 cm, stretched mesh size), and average weight of 75 g (Figure 2.3a) were placed in the experimental net-cages in Jaha River estuary (Figure 2.3b). The net multifilament had a mean diameter of 1.2 mm. The similar net panels but of 0.2 m x 2.5 m dimensions and average weight of 105 g were used in Sangga Besar River estuary where net-cage units were deeper. The lower end of each net panel was weighted down using lead sinkers to a vertical depth of 2 m (at Jaha) or 2.5 m (at Sangga Besar). The upper end was tied to an aluminium bar held horizontally across the net-cages wooden frame (Plate 2.4). In Jaha farm, the first 0 – 1.32 m of the net panel was vertically positioned, while the remaining part (1.32 – 2 m) gently curved over to follow the contour of the tapering net-cage bottom (Figure 2.3c). In Sangga Besar farm, the first 0 – 1.66 m of net panels was vertically positioned, while the remaining part (1.66 – 2.5 m) gently curved over to follow the contour of the tapering net-cage bottom. Four (dry season) or six (wet season) nylon panels each were placed on opposite sides of each cage unit in an alternate fashion to avoid overlap of their curved sections at the bottom of the

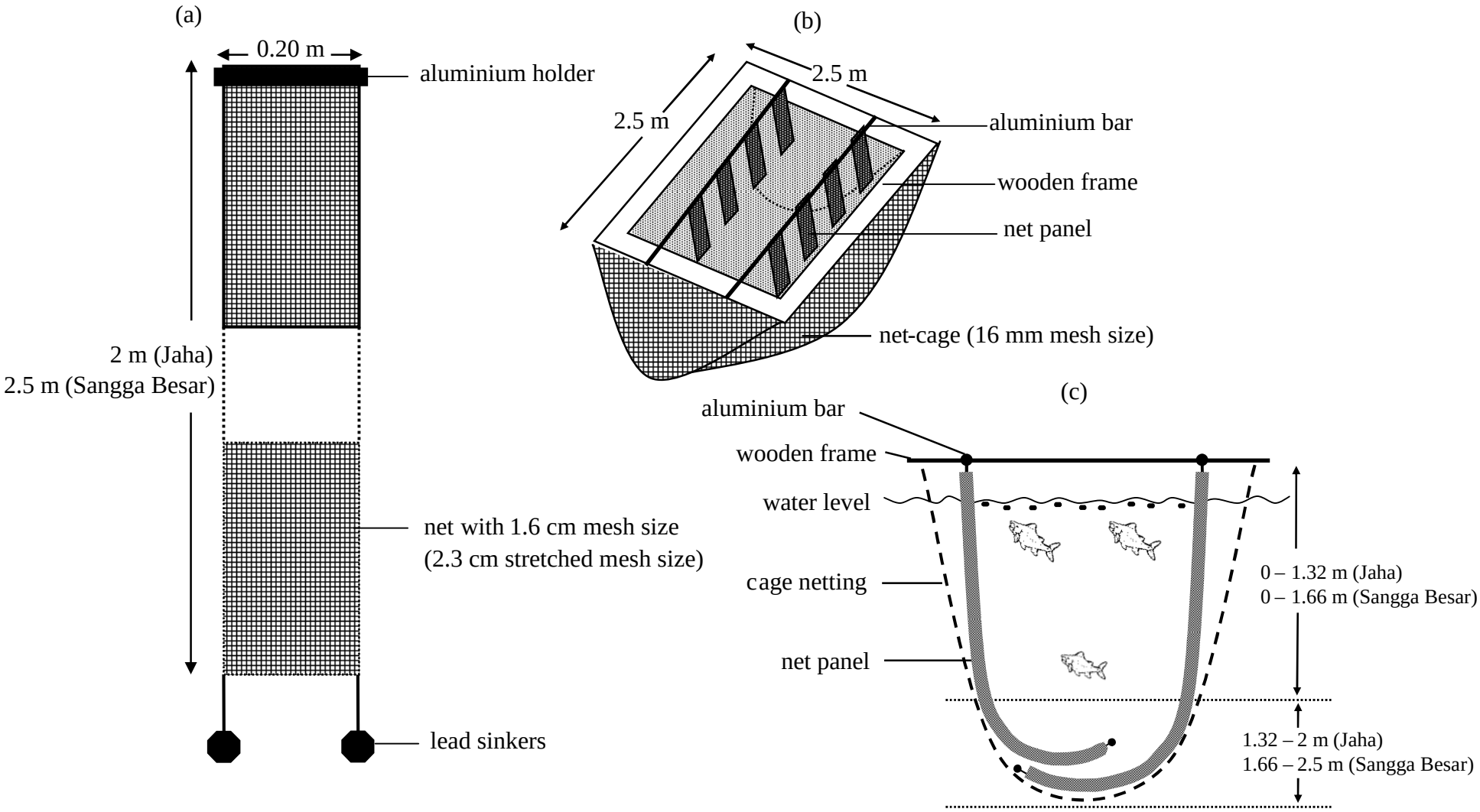


Figure 2.3. (a) Sketch diagrams of single experimental net panel; (b) how several net panels were positioned inside an experimental net-cage and (c) cross-section view of how the upper (0 – 1.32 m in Jaha &, 0 – 1.66 m in Sangga Besar) of the net panels was vertically positioned while the lower part (1.32 – 2 m in Jaha &, 1.66 – 2.5 m in Sangga Besar) gently bent over to follow the contour of the tapering net-cage bottom.

(ii)

Plate 2.4. Horizontal aluminum bars, inside (i) and outside (ii) were held across the net-cages to suspend the experimental net panels inside the net-cages.

cage.

Another set of triplicate net panels considered as 'non-caged' panels (O1, O2, O3) were placed outside the net-cages (i.e. outside farm), approximately 5 m distance from treatments P and T (see Figure 2.2a and 2.2b). Thus the macrofouling assemblages here represented organisms without the effects of fish rearing such as regular inputs of fish feed, fecal material and will experience strong water flow through them as compared to net panels placed inside the net-cages (P and T).

Macrofouling development on the pre-weighed net panels was then followed each week by gently removing one net panel from each experimental net-cage with the aid of a dip net (2.5 mm mesh size), until the completion of the experiment. The experiments were terminated at the end of the 8^{th} week for the dry season and the 12^{th} week for the wet season, that is, when the net meshes were completely occluded by macrofouling. Removed net panels with fouling organisms were immediately preserved in buffered 10% formalin, in separate 1-litre plastic jars.

In the laboratory, two types of analyses on biofouling organisms were carried out, first to study the biofouling biomass over time and the other to study the community structure of macrofouling assemblages such as species composition, abundance, colonization dynamics and including the depth distribution of sessile organisms on net panels.

2.2.1.2. Physical and Chemical Parameters

Salinity, turbidity, temperature, pH and dissolved oxygen (DO) were measured fortnightly at the surface (0.5 to 0.75 m depth) and net-cage bottom (1 to 2 m depth), using a YSI 3800 multiparameter sonde. The water parameters were recorded 2 hours before fish feeding, and before the experimental net panels were sampled. This was done

to reduce any interference from the active movement of cultured fish during and after feeding. Water velocity at the experimental net-cages was measured fortnightly at 0.5 to 0.75 m depth by a Toho Dentan electric current meter Model CM-2 (Toho-Dentan Co., Ltd., Tokyo, Japan) during flood flow (neap tide), 1 – 2 hours after low slack tide. Current velocity was measured at mode 'B' averaged over 20 seconds.

Daily rainfall data were obtained from the Department of Meteorology for rainfall over the Taiping Hospital, Perak, Malaysia located at Latitude 04° 52' N and Longitude 100° 44' E and height above M.S.L: 18.0 m. This rainfall station is located nearest (10 km eastward) to the MMFR. Data analysis of the rainfall data, indicated the 'dry season' from July – October 2000, while the 'wet season' occurred from November 2000 – March 2001.

2.2.1.3. Laboratory Treatment and Analysis

Laboratory work was carried out in the Environmental Laboratory, IPT, University of Malaya. Sessile forms consist of plants and animals while the non-sessile organisms are motile macrofauna. The sessile plant macrofoulers were completely sedentary but the animal group consisted of either sedentary or sessile forms. According to Hughes (2001), sessile fauna habitually remain in one location or capable of only very slow movement across the substratum, but not permanently attached to their site of larval settlement. Among the other characteristics of sessile or sedentary fouling organims are a free-swimming larval phase and a sedentary adult form that can firmly adhere to the substratum and extract dissolved nutrients or particulate material from the water column. The term 'sessile macrofouling' is used to refer to living organisms of either animals or plants which have settled, grown or developed colonies that are attached or encrusted on to the substrate surface. The sessile macrofouling organisms comprise of both calcareous and non-calcareous species, such as algae, hydroids, bryozoans, mussels and barnacles.

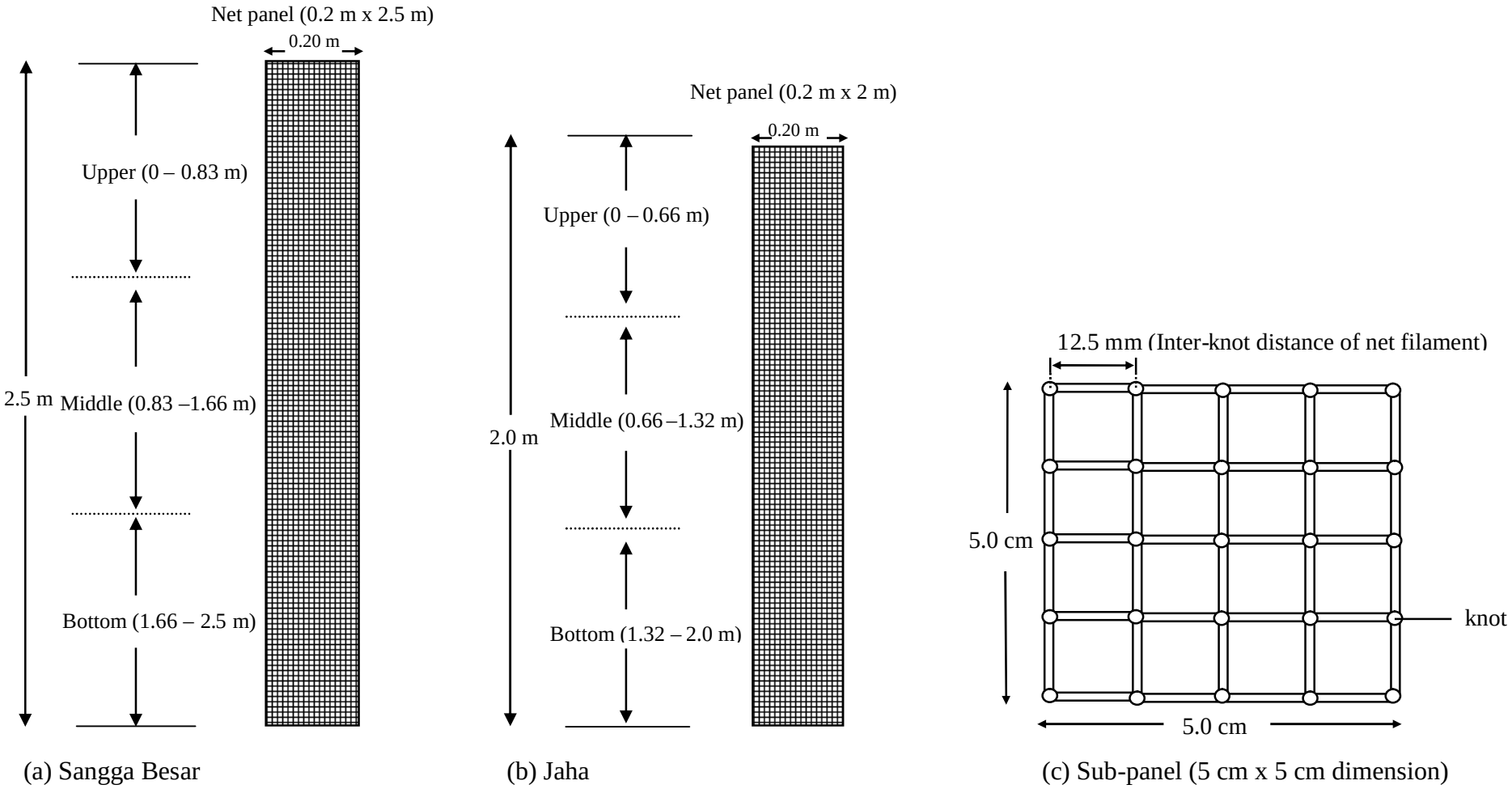
The term ‘non-sessile’ is used to refer to those motile, free-swimming organisms that live or hide amongst the sessile organisms on the net panels. They have the capability to swim, cling or live as tube dwellers on the surface of the net panel. They mainly consist of small macrofauna of either infauna or epifauna. Example of infauna species are those of burrowing nematodes and polychaetes. The epifauna species are above-substrate forms and tend to be larger, with the ability to swim for short distances and often present in great abundance (Hicks, 1977). Examples include isopods, amphipods, copepods, tanaids, etc.

2.2.1.3.1. Determination of Biofouling Total Wet Biomass

Each net panel was gently agitated, removed from its jar and weighed. The difference in weight before and after the experiment represented the weight (g per panel) of the sessile biofouling organisms on the net panel. The net panel with its sessile macrofoulers was then returned to its bottle with fresh buffered 5% formalin for further analyses. Non-sessile organisms, which had dropped to the bottom of the bottle after agitation, were collected by sieving the entire fluid through a 56 – 125 μm-mesh Endecott sieve and quickly rinsed with running tap water to remove fine sediments. The non-sessile organisms were then placed onto a preweighed wire gauze of the same mesh size, their combined wet weight (g per panel) was determined as a wet biomass of non-sessile organisms. Samples were immediately resuspended in 70% alcohol solution for further analyses.

2.2.1.3.2. Studies of Sessile Macrofouling Organisms

To investigate the depth distribution and abundance of sessile macrofoulers, the net panel was equally sub-sampled at three depth strata, upper (0.00 – 0.83 m), middle (0.83 –1.66 m) and bottom (1.66 – 2.50 m) at Sangga Besar (Figure 2.4a), while in Jaha the arbitrary depth strata were slightly different; upper (0.0 – 0.60 m), middle (0.60 – 1.32 m) and



(a) Sangga Besar (b) Jaha (c) Sub-panel (5 cm x 5 cm dimension)

Figure 2.4. Net panel with dimension of (a) 0.2 m x 2.5 m in Sangga Besar, (b) 0.2 m x 2.0 m in Jaha divided into three depth strata (Upper, Middle and Bottom) for analysis. "Bottom stratum" bent over net bottom (See Figure 2.3c). (c) Sub-panel of 5 cm x 5 cm dimension, comprising 40 net filaments with a 12.5 mm length and 1.22 mm diameter.

bottom (1.32 – 2.00 m) (Figure 2.4b). Three sub-panels of 5 cm x 5 cm area were randomly picked and cut out from each strata of the net panel (Figure 2.4c). To ensure random sampling, a random numbers table was used to pick a coordinate pair read as the node or intersection of the net grid (meshes). Stratified sampling was done to ensure equal samplings at different depth layers since certain species appeared to be preferentially distributed with depth.

Sessile biofoulers were quantified based on cover (%). Cover was determined by estimating the area occupied by the species under a stereo microscope. This procedure was carried out by simply estimating the occupied surface area (e.g. $^1/_4$, $^1/_2$ or $^1/_3$) of each net filament (or inter-knot distance of 12.5 mm), and finally summing up as the total number of filaments surface area occupied by the species. A fully occupied filament would give a score of 1 or one filament surface area occupied. The total number of filaments per 25 cm^2 sub-panel was 40 (see Figure 2.4c). The weekly total percentage covers of each sessile macrofouling species on net panels were determined for the colonization dynamics study.

The sessile macrofouling organisms were identified to the highest taxa possible under a stereo microscope. The algal species was confirmed by Professor Dr. Phang Siew Moi, Algal laboratory, IPT, UM. The following references were used to identify barnacles, mussels, bryozoans and hydroids: Chuang (1961); Barnes (1982); Shepherd & Thomas (1982); Higgins & Thiel (1988); Rupert & Barnes (1994); Carpenter & Niem (1998).

2.2.1.3.3. Studies of Non-Sessile Macrofouling Organisms

Non-sessile organisms or mobile organisms, which had dropped to the bottom of the bottle after agitation, were collected by sieving the entire fluid through a 125 μm-mesh Endecott sieve and quickly washing the retained organisms with running tap water to

remove off the fine sediments. They were then placed onto a pre-weighed wire gauze of the same mesh size, weighed and immediately resuspended in 70% alcohol solution (Figure 2.5). A Stempel pipette (0.5 ml) was used to obtain a homogeneous sub-sample (Plate 2.5), which was placed in a petri dish and observed under a Leica MZ8 microscope or if necessary a compound microscope. Sub-samplings were taken and species abundance was enumerated until no new species were encountered. Usually between 10 and 15 sub-samplings were done. Unlike sessile organisms, the abundance of non-sessile organisms or mobile macrofauna could not be defined by depth zonation but for the entire net panel. The non-sessile organisms were identified to the lowest taxa possible under a stereo microscope.

The Amphipoda were identified to the genus level by sending preserved specimens to Dr. Andreas Hughes, Hamburg University of Germany. The other non-sessile organisms such as tanaids, copepods, isopods, nematodes and polychaetes were identified using the following references: Barnes (1982); Higgins & Thiel (1988); Martin & Davis (2001); Day (1967a, b); Shepherd & Thomas (1982); Rupert & Barnes (1994). The details of this study are further presented in Chapter 3.

2.2.1.4. Computation and Statistical Analysis

The total wet biomass of the sessile biofouling organisms and non-sessile organisms on net panels was estimated as follows:

Biomass of sessile biofouling (g) = Weight of net panel before immersion (g) – Weight of net panel after immersion (g)

Biomass of non-sessile biofouling (g) = Weight of non-sessile associate from net panels (g) – Weight of wire gauze sieve used (g)

The percentage cover of the sessile macrofoulers on net panels was estimated as follows:

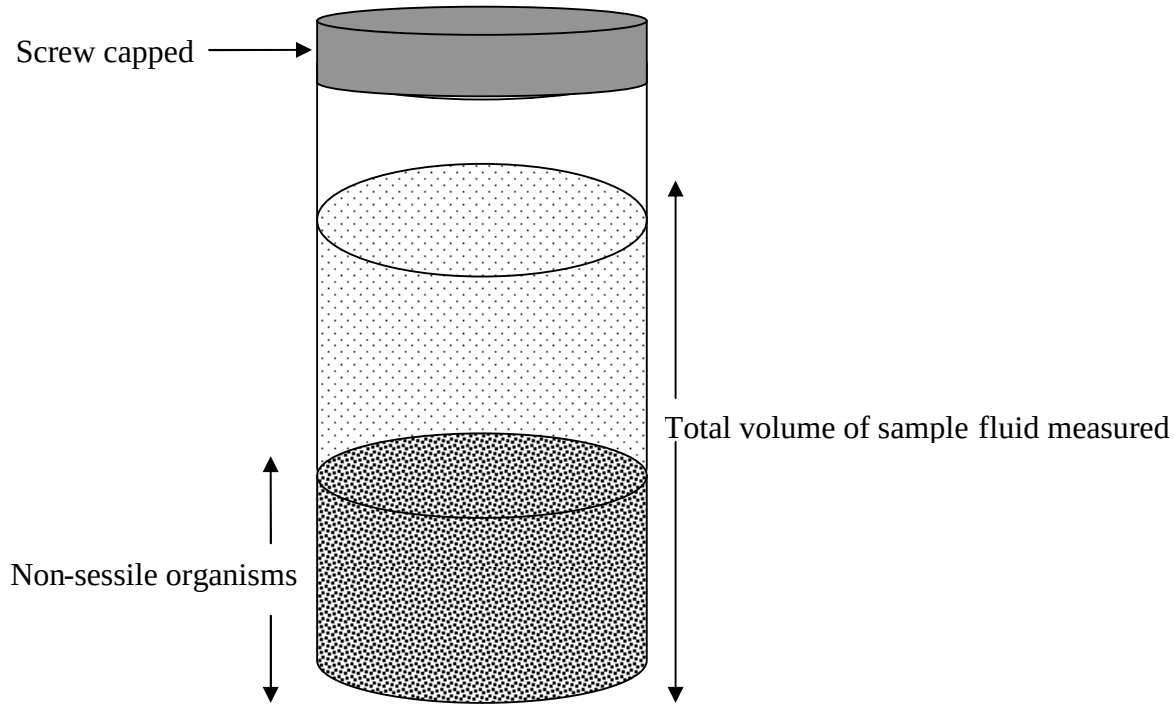


Figure 2.5. Non-sessile macrofouling organisms resuspended in 70% alcohol solution inside the screw capped bottle.

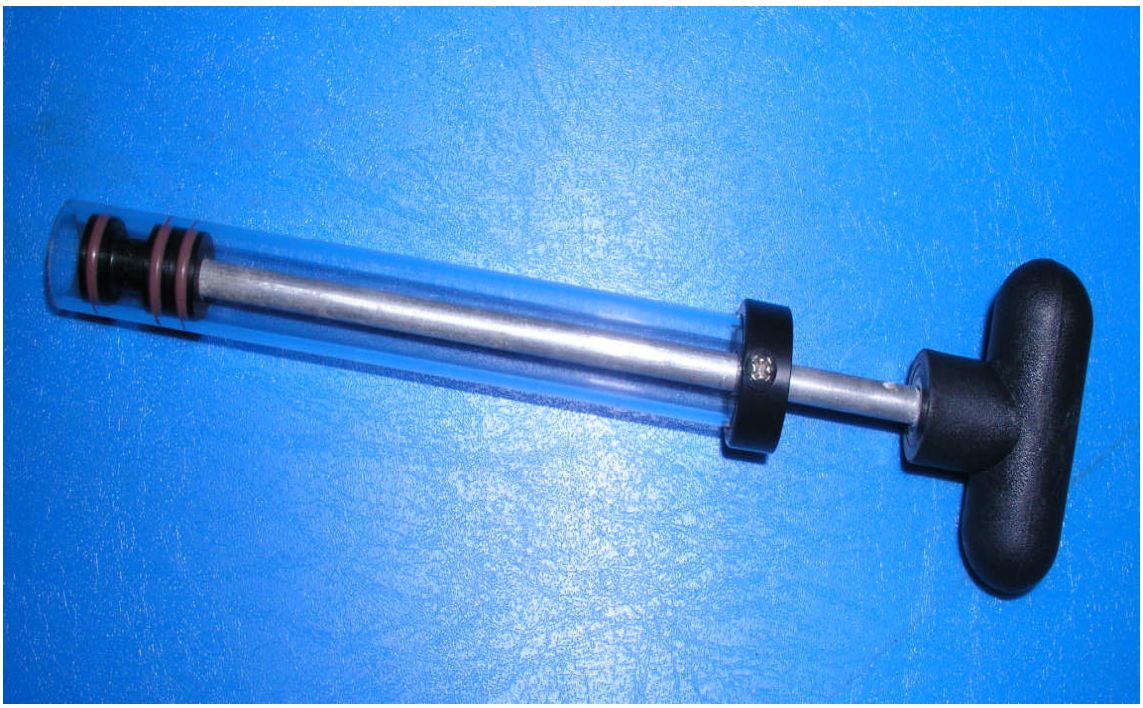


Plate 2.5. Stempel pipete (0.5 ml) was used to obtain a homogeneous subsample of non-sessile macrofouling organisms from the screw capped bottle (see Figure 2.5).

$$\textbf{Percentage cover (\%)} = \frac{\textbf{Total number of filament lengths occupied}}{\textbf{40}} \times 100$$

[Given that the total number of filaments per 5 cm x 5 cm (25 cm^2) of the sampled sub- panel was 40. Each net filament (12.5 mm length by 1.22 mm diameter) provided an attachment surface area of approximately 50.26 mm^2. See Figure 2.4c]

The density of non-sessile organisms (no. per 100 cm^2 or 1 dm^2 of net panel) was estimated as follows:

(a) Jaha River

$$\textbf{Density} = \frac{\textbf{Mean number of enumerated individuals}}{\textbf{0.5}} \times \frac{\textbf{Volume of sample fluid (ml)}}{\textbf{40}}$$

[Given that the Stempel pipette sampled 0.5 ml and the total area of net panel (20 cm x 200 cm) was 4,000 cm^2 or 40 dm^2]

(b) Sangga Besar River

$$\textbf{Density} = \frac{\textbf{Mean number of enumerated individuals}}{\textbf{0.5}} \times \frac{\textbf{Volume of sample fluid (ml)}}{\textbf{50}}$$

[Given that the Stempel pipette sampled 0.5 ml and the total area of net panel (20 cm x 250 cm) was 5,000 cm^2 or 50 dm^2]

2.2.1.4.1. Univariate Analysis

Computed percentage cover and density/biomass data were subjected to arcsine or logarithmic [$\log_{10}$ (x + 1)] transformations, respectively, so as to achieve normality and homogeneity of variance before statistical analysis (Zar, 1998). The logarithmic transformation of abundance data also has the effect of compressing the upper end of the measurement scale and reduces the importance of large values relative to smaller values in the data matrix (Digby & Kempton, 1996). A 3-factor ANOVA was carried out to investigate the effects of treatment (trash-fish, pellet, no feed), season (wet, dry) and immersion time (week 1, 2, 3… 12) on physical and chemical factors, biomass and density of sessile and non-sessile macrofoulers, while 4-factor ANOVA including depth (upper, middle, bottom) was carried out to examine the effects on percentage cover of sessile organisms. The Student Newman-Keuls test was used for multiple comparisons of

the means when the ANOVA was significant ($P < 0.05$). All the statistical analyses were carried out using STATISTICA Version 8 Software Program.

2.2.1.4.2. Multivariate Analysis

Principal components analysis (PCA) was carried out to study net colonization by the biofouling community as possibly influenced by the feed input, during the dry and wet seasons. Orloci's chord distances were computed instead of Euclidean distances so as to avoid the paradox problem associated with the latter when species abundance data are used (Legendre & Legendre, 1998; Legendre & Gallagher, 2001). Chord distances were computed from the species abundance data via a transformation program downloaded from; http://www.bio.umontreal.ca/casgrain/en/labo/transformations.html.

The program converted a matrix of species abundance in such a way that the Euclidean distances among rows of the transformed matrix were equal to the chord distances among rows of the original data matrix. PCA of the species abundance data was performed using the CANOCO ver. 4.02 software (ter Braak & Smilauer, 1998). A correlation biplot on the first two principal components axes was obtained. Details of this study are further presented in Chapter 3.

2.2.2. Study on the Effects of Fish Rearing, Fish Feed, Water Flow and Net-Cage Position on the Biomass of Biofouling Organisms (Chapter 4, page 166 – 189)

Two experiments were conducted, first, to determine whether the fish rearing, type of pellet feed and water flow had significant affect on biofouling biomass, and the second experiment was to determine the effects of net-cage position in the farm on biofouling in relation to the type of fish feed input. In the first experiment, two types of fish pellet were used, one, a stable, commercially-produced extruded pellet and the other, steamed home-made pelleted feed. This study was also to test the hypothesis that fish feed with

higher water stability and lesser fine particulate content, contributes to less biofouling of cage nettings than those of low water stability and high content of fine particulates. Less-stable formulated feed breaks up easily and their higher content of fine particulates provides more food to macrofouling organisms especially the filter feeders. This experiment did not use trash fish feed because it is also conducive to biofouling development as in home-made pellet feed. To study the additional effects of fish rearing and water flow, two control treatments were set up. The first control comprised net panels placed inside net-cages without fish and feed, and the second control comprised net panels placed outside net-cages. In the second set of experiment, nine net-cages were selected, 3 on the upstream end of the farm, 3 on the downstream and another 3 in between these locations or mid-position. The experimental layout of the different treatments was based on the Latin Square design. These experiments were conducted in a fish farm at Jaha River estuary (see Figure 2.1c).

2.2.2.1. Experimental Method and Sampling

The study was carried out from May – July 2005. In the first experiment, nine unfouled net-cages were deployed at the downstream end of the farm to investigate the effects of two types of pellet feed with different water stability and water velocity on net biofouling (Figure 2.6a). The experimental net-cages were in triplicates given the following treatments: (1) stocked fish given commercially-produced extruded pellet feed (M1, M2, M3), (2) stocked fish given home-made pellet feed (P1, P2, P3), and (3) no fish and feed (N1, N2, N3). Another treatment was located outside the net-cages and referred to as (4) negative control i.e. no fish, feed and enclosing netting (i.e. outside cage unit but within farm) (C1, C2, C3). The biofouling here represented the natural biofouling without the effects of fish rearing, i.e. the regular inputs of fish feed and fecal material. The C treatments outside the cages were expected to have higher water flow through

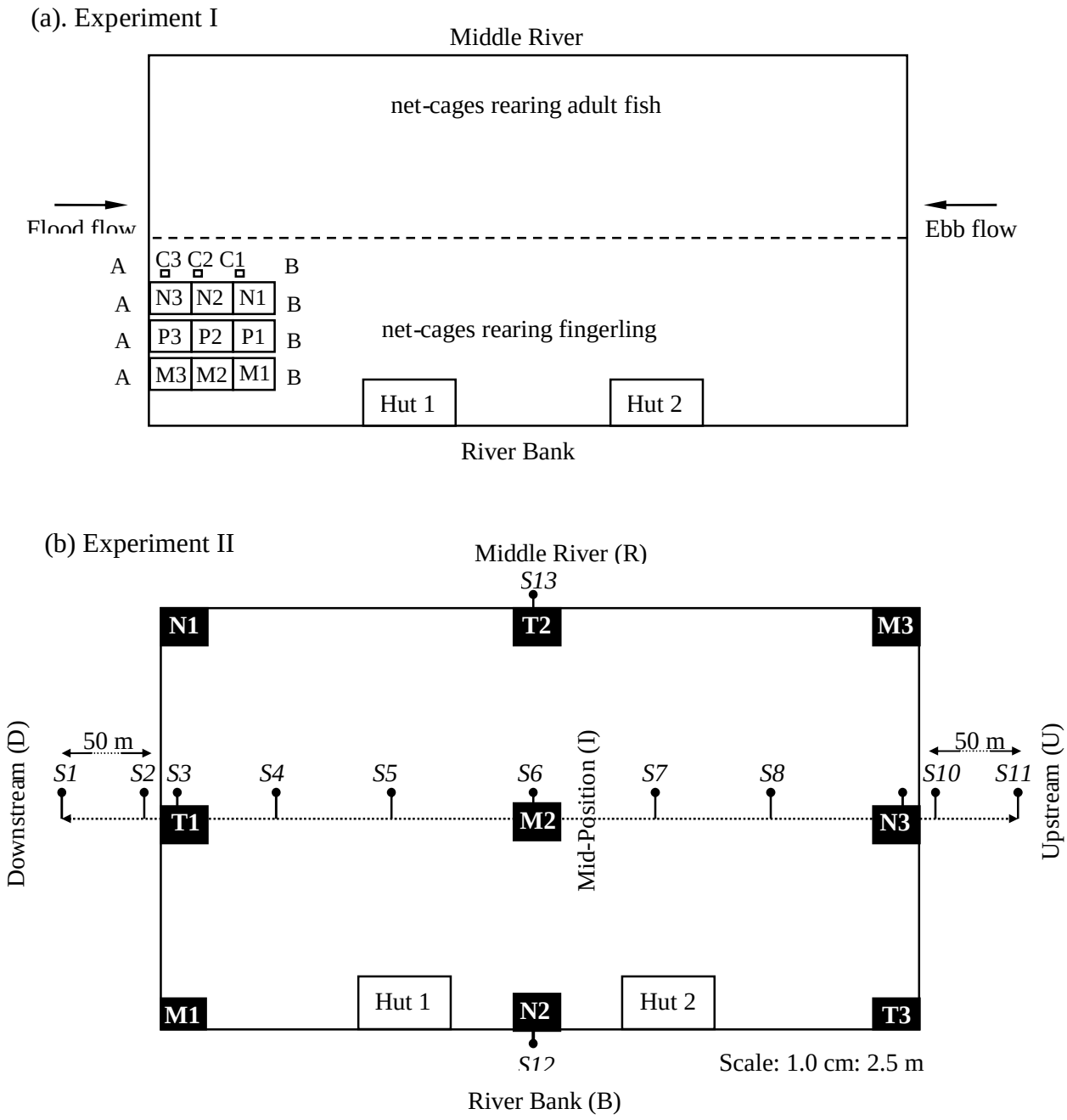


Figure 2.6. (a) Experiment I: Net-cage with fish given commercially-produced extruded pellet feed (M1, M2, M3); net-cage with fish given home-made pellet feed (P1, P2, P3), net-cage without fish and feed (N1, N2, N3); and without fish, feed and outside net-cage, control (C1, C2, C3). Net-cages at the near middle river direction were mainly used to culture matured fish while fingerlings reared at the near river bank direction of the farm, and (b) Experiment II: Allocated net-cages following Latin Square Design: net-cage with fish given commercially-produced pellet feed (M1, M2, M3); net-cage with fish given trash-fish (T1, T2, T3); and net-cages without fish and feed (N1, N2, N3). Physical measurements were made along transect D–I–U (dotted arrowed line), at stations S1 through S11. Additional stations were S12 (river bank) and S13 (middle of river).

them as compared to the M, P and N treatments inside the net-cages. To ensure that all experimental net-cages were exposed to the same or near similar water flow regime, net-cages were positioned at the downstream end of the farm. This selection was also to reduce cross-contamination during fish feeding carried out at low slack, but feed given were completely consumed by fish within the first 15 min.

The commercially-produced extruded feed pellets (Charoen Pokphand Feedmill Co. Ltd. Selangor, Malaysia), of 4 mm diameter, were composed of 40% protein and 0.4% lipid with maximum moisture of 12%. The home-made pellets (National Prawn Fry & Production Centre, Pulau Sayak, Kedah, Malaysia) were made from poultry offal meal at a mean pellet size of 4 mm diameter. Percentage contents of crude protein, crude lipid and ash based on dry weight were 42.5%, 12.6% and 13.1% respectively. Moisture content was 5.4%. Based on a simple stability test, the commercially-produced extruded pellets maintained stability without disintegration for 30 minutes as compared to the home-made pellet feed (P) which completely disintegrated. Both types of feed pellets were of the slow-sinking type with similar main ingredients (i.e. poultry offal meal). The experimental net-cages were initially stocked with 200 giant sea perch (*Lates calcarifer*) fingerlings (mean wet weight of 27 ± 4.5 g), the daily feed rates was 3 – 4% of the total biomass of stocked fish.

A second set of experiment was conducted four weeks thereafter to examine the effects of fish feed and net-cage position on biofouling biomass on nets. The net-cage position is the location of the cage unit along and across the water channel; it will primarily determines the flow rate. Its layout was based on a Latin Square Design with one and only one treatment replicate on each row and column. Nine, non-fouled net-cage units were deployed; three on the upstream end of the farm, three on the downstream end and three between these locations, i.e. mid-position (Figure 2.6b). Each block contained

3 net-cages and each net-cage received either commercially-produced extruded pellet feed (M), trash-fish feed (T) or no feed (N). Only M and T cages were each stocked with 100 giant sea perch fingerlings which were fed daily. The design thus examined the effects of fish feed treatment (M, T and N) along the longitudinal (D–I–U) and cross-river (R–I–B) axes of the farm.

To monitor the increment of biofouling biomass, the same procedure as described in section 2.2.1.1. was adopted (see also Figure 2.3).

Thus, in Experiment I, both P and M panels differed from N and C panels in term of regular exposure to fish feed and feces, whereas N and C were not. The difference between N and C net panels was that the latter will experience stronger water flow through them as compared to the former in a flow-reduced environment. In Experiment II, net panels sited at the mid-position of the farm were expected to experience slower water flow as compared to those on the outer perimeter. Net panel fouling biomass in both experiments was measured every week for 8 weeks, by removing one net panel from each experimental cage or outside it each week. The removed net panels were immediately immersed in buffered 10% formalin in separate 1-litre jars.

2.2.2.2. Physical and Chemical Parameters

In Experiment I, water parameters such as salinity, pH, dissolved oxygen (DO) and turbidity, were measured fortnightly at the surface (0.5 – 0.75 m depth) and net-cage bottom (1 – 1.5 m depth), using a Hydrolab DataSonde 4a Water Quality Multiprobes (Hydrolab Corporation, Texas, USA) during the flood and ebb flow for each treatment M, P, N and C. The water parameters were recorded 2 hours before fish feeding.

The water flow attenuation through the net-cages was measured from downstream (position A) through net-cages 3, 2 and 1 to upstream (position B) during flood flow of

(neap tide), 1 – 2 hours after low slack (see Figure 2.6a) at a depth of 0.5 – 0.75 m by a Toho Dentan electric current meter Model CM-2. The percentage of water flow attenuation on transmission through the series of net-cages in Experiment I was determined as follow:

$$\textbf{Water flow attenuation (\%)} = \left[\frac{\textbf{(Velocity at position A) – (Velocity in experimental net-cage)}}{\textbf{(Velocity at position A)}}\right] \times 100$$

In the second experiment, water parameters were additionally measured along a transect (D–I–U) of 13 sampling stations established across the entire farm. Sampling stations S1, S2, S10, S11, S12 and S13 were located outside the farm, whereas stations S3, S4, S5, S6, S7, S8 and S9 were located inside the farm with varying distance of 5 – 7.5 m between adjacent stations (see Figure 2.6b). Outside the farm, measurements at station (S1, S2, S10, S11, S12 and S13) were taken at both surface (0.5 – 0.75 m depth) and bottom (2.5 – 3 m depth). Within the farm (S3, S4, S5, S6, S7, S8 and S9), measurements were taken at three positions per station, i.e. surface, inside net-cage (0.5 – 0.75 m depth); surface, outside net-cage (0.5 – 0.75 m depth); and bottom, outside net-cage (2.5 – 3 m depth). All measurements were made during the flood and ebb flow of neap tide.

2.2.2.3. Laboratory Treatment and Analysis

In the laboratory, each net panel was gently agitated, removed from its jar and weighed. The agitation was to remove non-sessile organisms for later examination. The difference in weight before and after the experiment represented the weight (g per panel) of the sessile biofoulers. Non-sessile organisms, which had dropped to the bottom of the bottle after agitation, were collected by sieving the entire fluid through a 125 µm-mesh Endecott sieve and quickly washed with running tap water to remove fine sediments. They were then placed onto a preweighed wire gauze of the same mesh size, blotted dry

before their combined wet weight (g per panel) was determined. See Section 2.2.1.3.1. for details.

2.2.2.4. Computation and Statistical Analysis

The total wet biomass of the sessile biofouling organisms and non-sessile organisms on was estimated as in section 2.2.1.4.. Computed biomass data were subjected to logarithmic [$\log_{10}$ (x + 1)] transformation, to achieve normality and homogeneity of variance before statistical analysis (Digby & Kempton 1996; Zar, 1998). For Experiment I, a 2-factor ANOVA was carried out to investigate the effects of treatments (P, M, N, C) and immersion time (week 1, 2, 3… 8) on biofouling biomass of sessile organisms and non-sessile organisms. If the ANOVA is significant ($P < 0.05$), the Student Newman-Keuls test was used for multiple comparisons of the means. For Experiment II, the biofouling biomass values among block treatments (feed, longitudinal location, cross-river location) on a Latin Square design were analyzed for significant differences each week, for sessile organisms and non-sessile organisms. This study is further elaborated in Chapter 4.

2.2.3. Study on the Effects of Salinity on Macrofouling Community Structure (Chapter 5, page 190 – 205)

This study was conducted to test the hypothesis that higher salinity (as observed during the dry season) is more suitable for marine biofouling organisms and thus increases the biofouling rate; conversely, low salinity (as observed during the wet season) decreases the biofouling rate.

2.2.3.1. Experimental Method and Sampling

Live biofouling organisms were obtained in a fish farm at Jaha River estuary. Three net-cages given trash-fish feed were selected to obtain live biofouling organisms (Figure

2.7a). Five net panels of 0.2 m x 2 m dimension (Figure 2.7b) were placed inside the experimental net-cages to allow biofouling organisms to settle and develop on them (Figure 2.7c). All net panels with their biofouling loads were gently removed after 2 weeks. Removed net panels with their living biofouling organisms were kept inside large plastic bags containing aerated seawater and brought back to the laboratory. The water temperature was kept cool by ice cubes placed outside the bags to reduce stress on the biofouling organisms.

2.2.3.1. Laboratory Treatments and Analysis

In the Marine Culture Laboratory, three net panels were selected for each water tank containing seawater of 10 ppt, 15 ppt, 20 ppt, 25 ppt and 30 ppt salinity respectively (Plate 2.6a). Each water tank was prepared with condition such water flow and dissolved oxygen concentration that were as close as possible as in the field environment (Plate 2.6b). The net panel was inspected carefully to ensure that dominant species such as *Plumularia* sp., sea anemone, *Balanus amphitrite*, *Polysiphonia* sp., *Enteromorpha clathrata*, *Xenostrobus mangle* and *Cryptosula* sp. were all present on each net panel. This is important since the coverage of biofouling organisms on each net panel was slightly different despite a similar immersion period inside the net-cages. This was also to ensure that the composition of biofouling organisms were similar for the tested salinities.

Each net panel was cut into three strips according to depth strata: upper, middle and bottom (Figure 2.8a). Each depth stratum (with its different dominant species) was then cut into 3 smaller sub-panels of 22.22 cm x 20 cm dimension (Figure 2.8b). A total of nine sub-panels were obtained from each net panel. The sub-panels were placed inside the water tank to allow the biofouling organisms to grow under the different salinities (Figure 2.8c). A total of 27 sub-panels from three different net panels were tested for each salinity in the water tank. The biofouling organisms were fed daily with home-

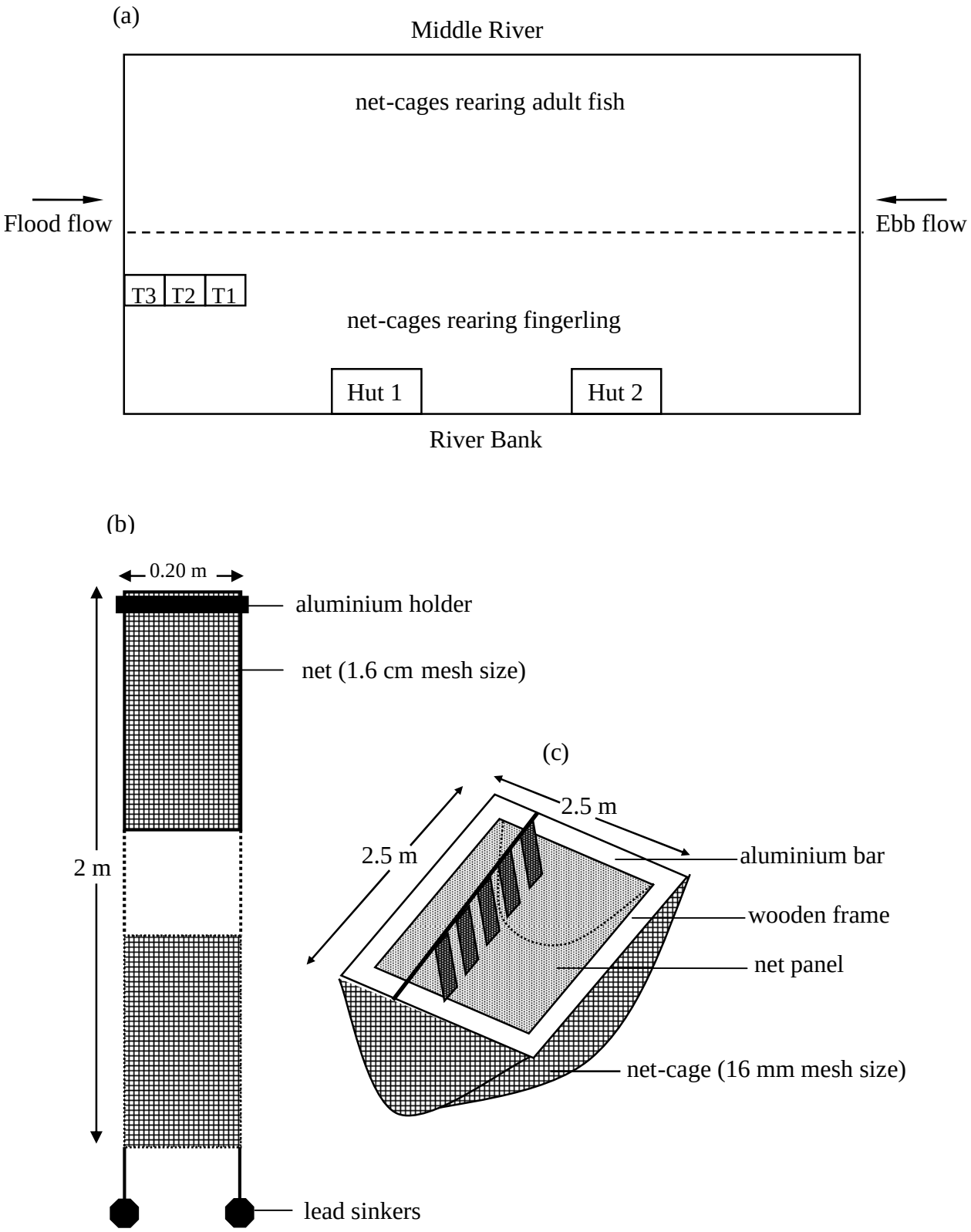
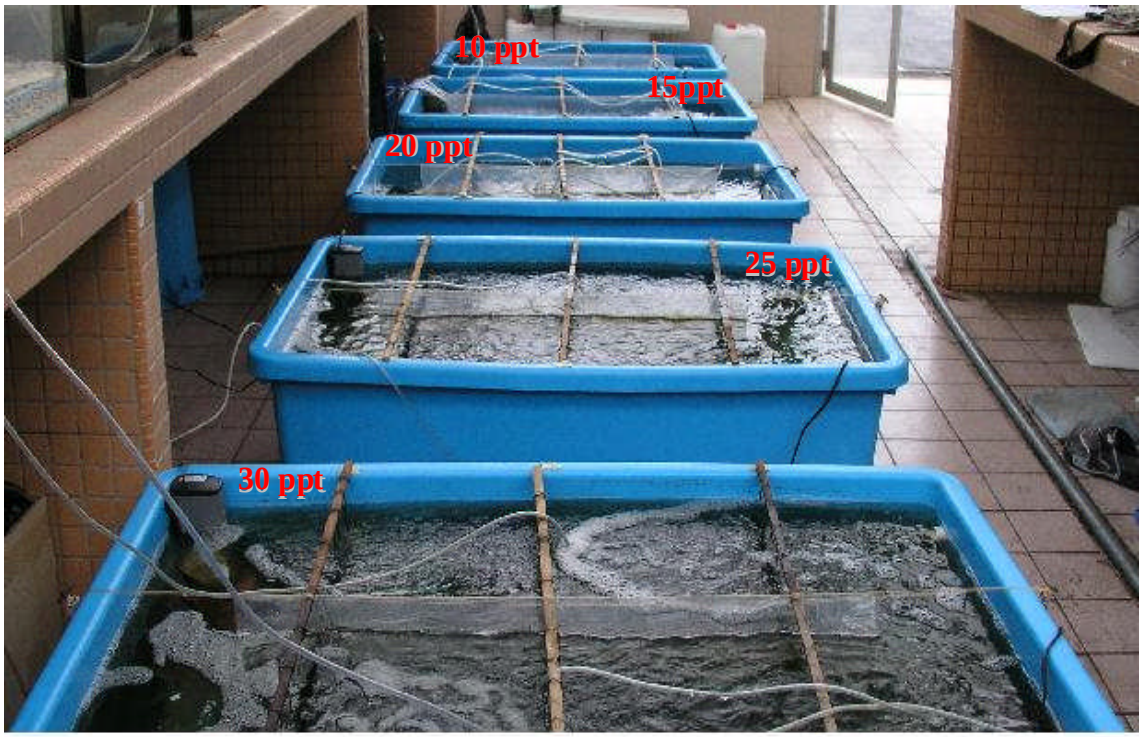


Figure 2.7. (a) Sketch diagrams of Jaha estuary fish farm, showing net-cages T1, T2 and T3 which were selected to grow and obtain 2-week old live biofouling organism on nets panels. (b) Net panel of 2.0 x 0.2 m dimension, and (c) how the five net panels were positioned inside the net- cages.

(a)



(b)

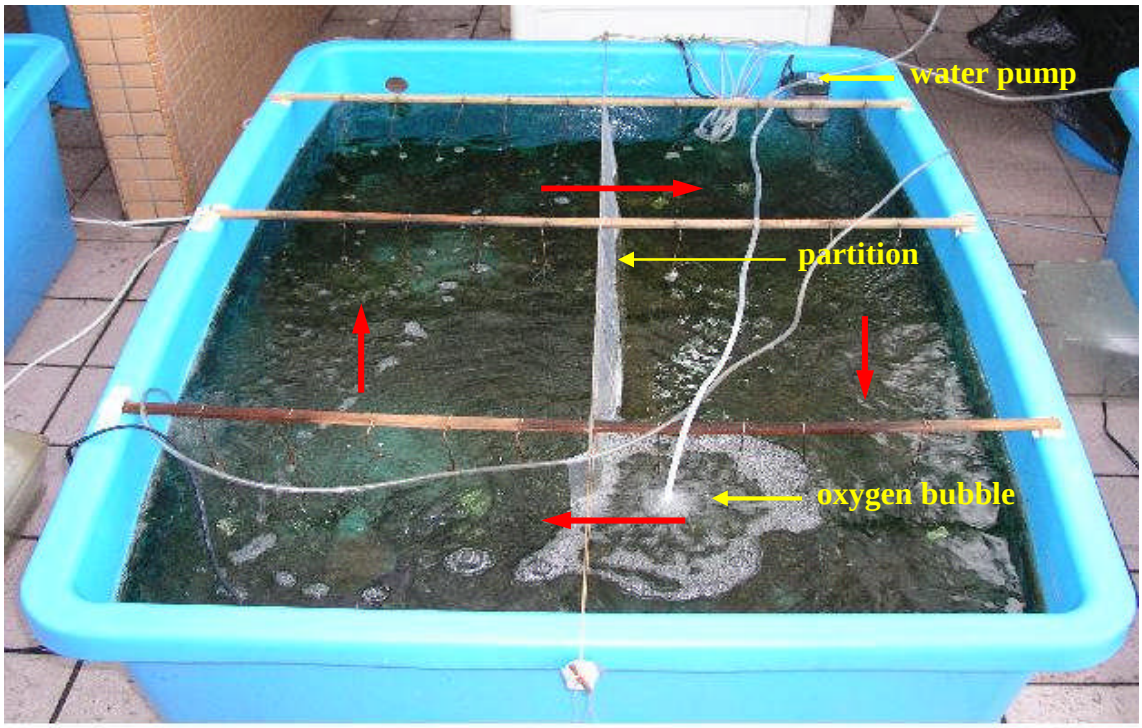


Plate 2.6. (a) Water tank with different salinity, 10 ppt, 15 ppt, 20 ppt, 25 ppt and 30 ppt respectively, and (b) a single water tank with several modifications, red arrows indicate a water flow movement.

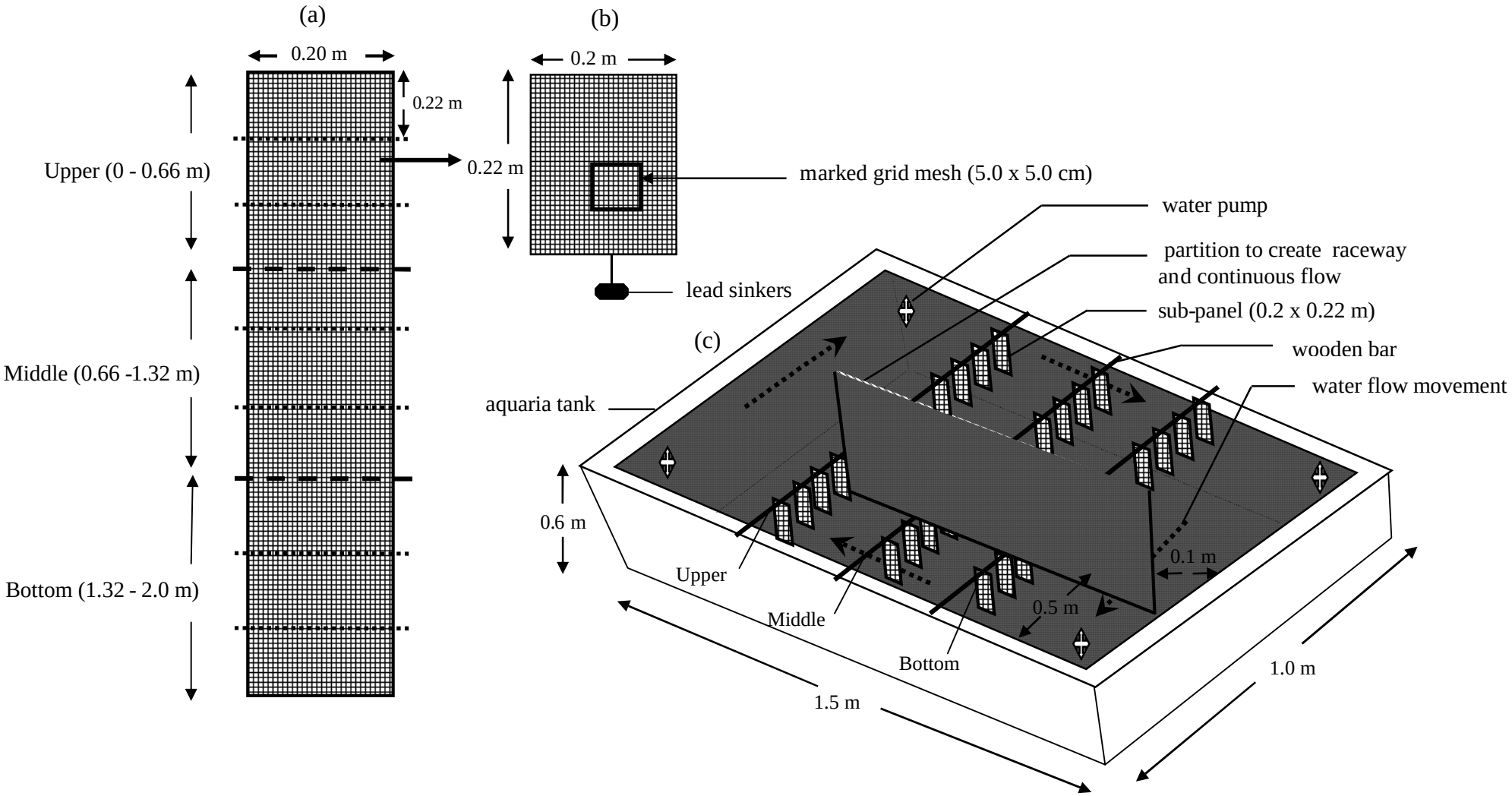


Figure 2.8. (a) Net panel of 2.0 x 0.2 m dimension with three depth strata, upper, middle and bottom respectively; each stratum was cut into three smaller sub-panels (b) each of 22.2 x 20 cm with marked grid of 5 cm x 5 cm dimension comprising 40 filaments, which were then positioned inside a water tank (1.5 x 1.0 x 0.6 m, dimension) as shown in (c) where 9 sub-panels each from upper, middle and bottom strata were suspended.

made pellet, ground trash-fish and commercial coral feed.

The salinity at the fish farm at the time of sampling varied between 16.21 ppt – 17.27 ppt. The seawater condition inside the experiment tanks were monitored and maintained daily to ensure that salinity, dissolved oxygen and pH were stable. Dissolved oxygen concentrations were maintained at above 50% saturation. This was done by adjusting the amount of aeration. pH was maintained at the range of 6 – 8, similar to the field condition. Salinity and pH were maintained by topping up with distilled water upon evaporation. Water flow and direction were created and controlled by electric water pumps (see Plate 2.6b and Figure 2.8c). Water flow was maintained at slow rate of <10 cm s^{-1} similar to the condition inside the net-cages in the estuary.

2.2.3.2.1. Study on the Development and Survival Rates of Sessile Macrofouling Species

Based on results of the first study (Chapter 3), sessile macrofouling organisms on net panels were abundant at particular depth stratum. Thus, for the purpose of this experiment development rates of macrofouling species were determined at the depth they developed best. For example, *Polysiphonia* sp., *Enteromorpha clathrata* and *Xenostrobus mangle* were analyzed for the upper stratum. Sea anemones and *Cryptosula* sp. for the middle stratum and *Plumularia* sp. and *Balanus amphitrite* for the bottom stratum.

Biofoulers development was monitored by estimating the percentage cover occupied by fouling species. Squares of 5 cm x 5 cm were randomly marked with white nylon string on each sub-panel (see Figure 2.8b). To ensure random sampling, a random numbers table was used to pick a coordinate pair read as the node or intersection of the net grid (meshes). Stratified sampling was done to ensure equal samplings at different

depth layers since certain species appeared to be preferentially distributed with depth. The development of sessile macrofoulers were quantified based on cover (%). Cover was determined by estimating the area occupied by the species under a stereo microscope. The procedure as described in Section 2.2.1.3.2..

The marked sub-panel was placed on a enamel tray with appropriate volume of sea water for enumeration and returned into the water tank after enumeration. The estimation of cover was done quickly to avoid stress on the organisms. The coverage of each biofouling species was estimated weekly until at the 3rd week or terminated when there were no more growths (i.e. no changes on colony size) or when all organisms died. For the purpose of this experiment, biofoulers such as barnacles, mussels and antozoans were carefully observed under a low power zoom microscope to determine whether they were still alive or dead. For example, barnacles (*Balanus amphitrite*) were considered dead when their shells were opened and empty inside. Mussels (*Xenostrobus mangle*) were considered dead when their shell was permanently opened while anemones were considered dead when their tentacles or polyps was permanently opened or with no immediate responses after gentle prodding.

2.2.3.3. Computation and Statistical Analysis

The percentage cover of the sessile macrofouling species was estimated as described in Section 2.2.1.4.. Computed percentage cover data were subjected to arcsine transformations to achieve normality and homogeneity of variance before statistical analysis (Zar, 1998). Repeated measure ANOVA was then carried out to investigate the effects of salinity (10 ppt, 15 ppt, 20 ppt, 25 ppt and 30 ppt) and immersion time (week 0, 1, 2… 3) on the total percentage cover of each macrofouling species. If the ANOVA was significant ($P < 0.05$), the Student Newman-Keuls test was used for multiple comparisons of the means. Dunnett test was carried out to investigate if there was any

significant changes in total percentage cover of each macrofouling species between week 0 (as control group) with week 1, 2 and 3. This study is further elaborated in Chapter 5.

2.2.4. Study of Nutrient and Chlorophyll-*a* Concentrations of Culture Water in Relation to Biofouling Development (Chapter 6, page 206 – 226)

2.2.4.1. Experimental Method and Sampling

This study was conducted to investigate whether there is a relationship between biofouling development with nutrient concentration and chlorophyll-*a* concentration of culture water inside the net-cages. The 12-hour study was conducted during the neap tide in a fish farm at Jaha River estuary during the dry season (14 October 2001). Six non-fouled net-cages at the downstream end of the farm were selected and set up in triplicates to receive either ground trash-fish (T1, T2 and T3) or pellet feed (P1, P2 and P3) (Figure 2.9). This position was selected to ensure that all experimental net-cages were exposed to the same or near similar current regime. This position also reduced cross-contamination from the upstream during fish feeding which was carried out at high slack tide, although feed given were completely consumed by fish within the first 15 minutes.

Home-made dry pellet and ground trash-fish feed were given separately to the selected experimental cage. Water samples were collected from each treatment before fish feeding (time 0) and after the fish feeding or at least after 30 minutes. Samples were collected at 30 minute intervals for 2 hours. Three more samples (O1, O2, O3) were taken approximately 5 m outside the farm (see Figure 2.9).

The above sampling procedures were conducted for flood, slack and ebb water. Samples of flood water were taken at 8.30 am – 10.30 am, followed by high slack water near or at 11.00 am – 1.00 pm and during ebb at 1.15 pm – 3.15 pm. Surface water samples were taken using a clean plastic bucket and then pour them into acid washed polyethylene bottles (250 ml) which were screw-capped, labeled and stored in an ice-

chest for subsequent analysis.

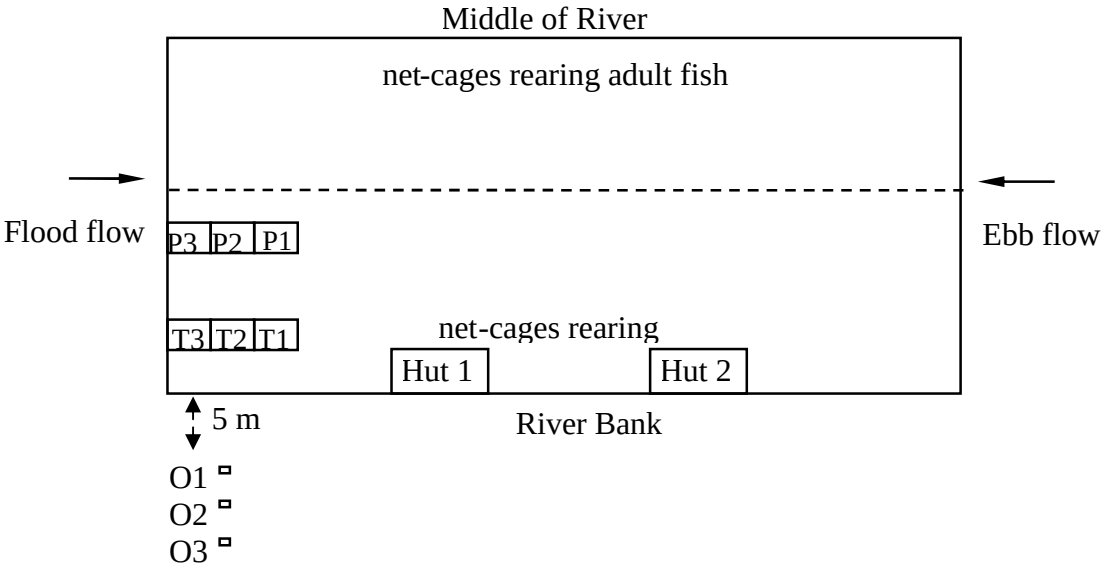


Figure 2.9. Sketch diagram of experimental treatments at the Jaha estuary farm. Samplings were conducted at P1, P2, P3: Home-made pellet feed; T1, T2, T3: Trash-fish feed and O1, O2, O3: Outside the net-cages.

Upon arrival on shore, the seawater samples were allotted for measurement of dissolved inorganic nutrients (NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}) and chlorophyll-*a*. A 100 ml of water sample was filtered through GF/C Whatman glass microfiber filter paper for the analysis of chlorophyll-*a* concentration. A few drops of 1% $MgCO_3$ solution were dropped onto the filtered paper containing phytoplankton cells which was then folded to appropriate size and wrapped in aluminium foil to prevent the breakdown of chlorophyll-*a* by light. Each wrapped filtered sample was individually kept inside a screw-capped opaque disc, labeled and stored in the freezer until laboratory analysis.

The remaining water samples were filtered through GF/C Whatman glass microfiber filter paper into acid-washed plastic bottles for dissolved nutrient analysis.

2.2.4.2. Physical and Chemical Parameters

Salinity, turbidity, temperature, pH and dissolved oxygen (DO) were measured at the surface (0.5 – 0.75 m depth), using a YSI 3800 multiparameter sonde. Water velocity

was measured at 0.5 – 0.75 m depth by a Toho Dentan electric current meter (Model CM-2). Water parameters were recorded before feeding at time 0 and after fish feeding or as soon as water samples were taken for each tidal phase.

2.2.4.3. Laboratory Treatment and Analysis

2.2.4.3.1 Nutrient Concentration

Dissolved nutrient concentrations of NH_3-N, NO_3-N, NO_2-N and PO_4^{-3} were determined by a HACH DR/ 2010 spectrophotometer. Step-by-step procedures were provided in the HACH Water Analyses Handbook (1997). Results in the unit of mg l^{-1} were converted into µmol l^{-1}. A reading obtained for NH_3-N, NO_3-N and NO_2-N were divided by the molecular weight of nitrogen (N = 14) while reading for PO_4^{-3} were divided by the molecular weight of ion PO_4^{-3} (PO_4^{-3} = 95).

2.2.4.3.2. Chlorophyll-a Concentration

Chlorophyll-*a* concentration were determined based on the fluorometric methods (Strickland & Parson, 1972; 1984). The filter paper containing the phytoplankton was torn into small pieces and put into a polypropylene test tube. 10 ml of 90% acetone and a few drops of 1% $MgCO_3$ were added into the tube for chlorophyll-*a* extraction. The phytoplankton cells together with pieces of filter paper inside the tube were repeatedly crushed with a glass rod until a very fine sample was obtained. Processed samples were screw-capped and stored in a refrigerator at 4^oC for 24 hours to allow complete extraction.

After extraction the containing tubes were taken out and spun in a centrifuge at 3,000 rpm for 10 minutes. The concentration of chlorophyll-*a* was then measured by a Turner Quantech fluorometer based on a predetermined standard curve programmed into the fluorometer. Blank sample of 90% acetone was measured and all reading was re-adjusted

with the blank sample reading.

The standard curve of chlorophyll-*a* was established based on a high but known concentration of chlorophyll extracted from a fresh culture of *Chlorella* sp. measured using a Shimadzu UV-VIS spectrophotometer at three different wavelengths, 665 nm, 645 nm and 630 nm. The concentration of chlorophyll-*a* in the solution was calculated based on Strickland & Parsons (1968) equation:

C =11.6 x OD665 – 1.31 x OD645 – 0.14 x OD630

Where OD = the absorbance at different wavelength

C = concentration of chlorophyll-*a* in (mg/ml)/10^3 = µg/ml

Concentration of chlorophyll-*a* in µg l^{-1} was calculated based on the following equation:

$$\textbf{Chlorophyll-}a\ (\mu g\ l^{-1}) = \frac{\textbf{C x 10 ml of extracted sample}}{\textbf{100 ml of filtered water sample x 1000}}$$

2.2.4.4. Statistical Analysis

Computed data of nutrients and chlorophyll-*a* concentration were subjected to logarithmic [$\log_{10}$ (x + 1)] transformations, respectively, so as to achieve normality and homogeneity of variance before statistical analysis (Digby & Kempton, 1996; Zar, 1998). Repeated measure ANOVA was carried out to investigate the possible effects of treatments feed (trash-fish, home-made pellet, outside net-cage 'control'), and interval time (minutes 0, 30, 60… 120) on the concentration of dissolved nutrients and chlorophyll-*a* during each tidal phase. The Student Newman-Keuls test was used for multiple comparisons of the means. Dunnett test was carried out to investigate if there was any significant change in nutrients and chlorophyll-*a* concentration before feeding at minutes 0 (control group) and after feeding at 30, 60… 120 minutes. This study is further elaborated in Chapter 6.

2.3. Limitations of Study

This study used net panels of identical mesh material, mesh size and filament thickness as the nettings of the cage-units which enclosed them (see Figure 2.3). The hung net panel was meant to simulate as close as possible the cage-unit netting as a substrate for biofouling. It is however not exactly identical in contour but the conditions inside the cage unit were the exact conditions for the hung panel. The requirement for weekly monitoring of biofouling biomass including community structure, concomitant with fish rearing, necessitates such a methodology which would allow the random samplings of similar net panels week after week. Thus, it used a completely randomized design and the sampled net panels were assumed to be independent of each other. A repeated measures design, i.e. monitoring of the same panel week after week, would have been statistically more powerful and realistic, but its benefits would have been offset by the repeated disturbance of sampled fauna (e.g. when out of water) and sampling of non-sessile organisms would displace or leave none behind for the next sampling. On the other hand, the completely randomized design has none of these problems except that future population could sometimes be less than past population due to sampling and uneven growth. However, replications (including stratified samplings for the community analysis) reduced this problem to a good extent.

2.4. Summary of Experimental Designs Used and Analysis

Summarized experimental design used and analysis were presented in Table 2.1..

Table 2.1. Summary of experimental design and analysis used in the study.

Study	Design	Analysis	Testing situation
1) Community Structure, short-term colonization dynamics and biomass of macrofouling	Completely randomized design with equal replication. Factors involved:	*Univariate Analysis:* A 3-factor ANOVA (Treatment feed* Season * Immersion time) on physical &	1 & 2 (Figure 2.10)

assemblages on nets (Chapter 3).	*i.* Treatment feed (T, P, O). *ii.* Season (dry, wet). *iii.* Immersion time (week 1, 2, 3…12).	chemical factors and biomass, percentage cover and density of sessile and non-sessile macrofoulers. The Student Newman-Keuls test was used for multiple comparisons of the means. *Multivariate Analysis:* PCA to study net colonization by the biofouling community as possibly influenced by the feed input, during the dry and wet seasons.	
2) Effect of fish rearing, fish feed, water flow and net-cage position on fouling biomass. (Chapter 4)	Experiment I: Completely randomized design with equal replication. Factors involved: *i.* Treatments feed (P, M, N, C). *ii.* Immersion time (week 1, 2, 3…8). Experiment II: 3 X 3 Latin Square Design Factors involved: *i.* Treatments feed (M, T, N). *ii.* Longitudinal position (D, I, U). *iii.* Cross-river position (R, I, B).	A 2-factor ANOVA, (Treatments feed* Immersion time), on biofouling biomass. The Student Newman-Keuls test was used for multiple comparisons of the means. ANOVA: (Feed * Longitudinal position * Cross-river position) on biofouling biomass each week.	1, 2 & 3 (see Figure 2.10) 1 & 3 (see Figure 2.10)
3) Effects of salinity on macrofouling community structure (Chapter 5).	A Repeated Measure Design. Factors involved: *i.* Salinity (10, 15, 20, 25, 30). *ii*. Immersion time (week 0, 1, 2, 3).	A 2-factor Repeated Measure ANOVA, (Salinity * Immersion time) on percentage cover of sessile biofouling organisms. The Student Newman-Keuls test was used for multiple comparisons of the means. Dunnett test to investigate any significant changes between control group i.e. at week 0 and subsequent week i.e. 1, 2 & 3.	No testing situation involved

4) Nutrient and chlorophyll-*a* concentration of fish culture water in relation to biofouling development (Chapter 6).	A Repeated Measure Design. Factors involved: *i.* Treatment feed (P, T, O). *ii.* Interval time (minute 0, 30, 60, 90, 120).	A 2-factor Repeated Measure ANOVA, (Treatment feed * Interval time) on nutrient and chlorophyll-*a* concentrations. The Student Newman-Keuls test was used for multiple comparisons of the means. Dunnett test to investigate any significant changes between control group i.e. at minute 0 and subsequent time i.e. 30, 60, 90 & 120 minutes.	1 & 2 (see Figure 2.10)

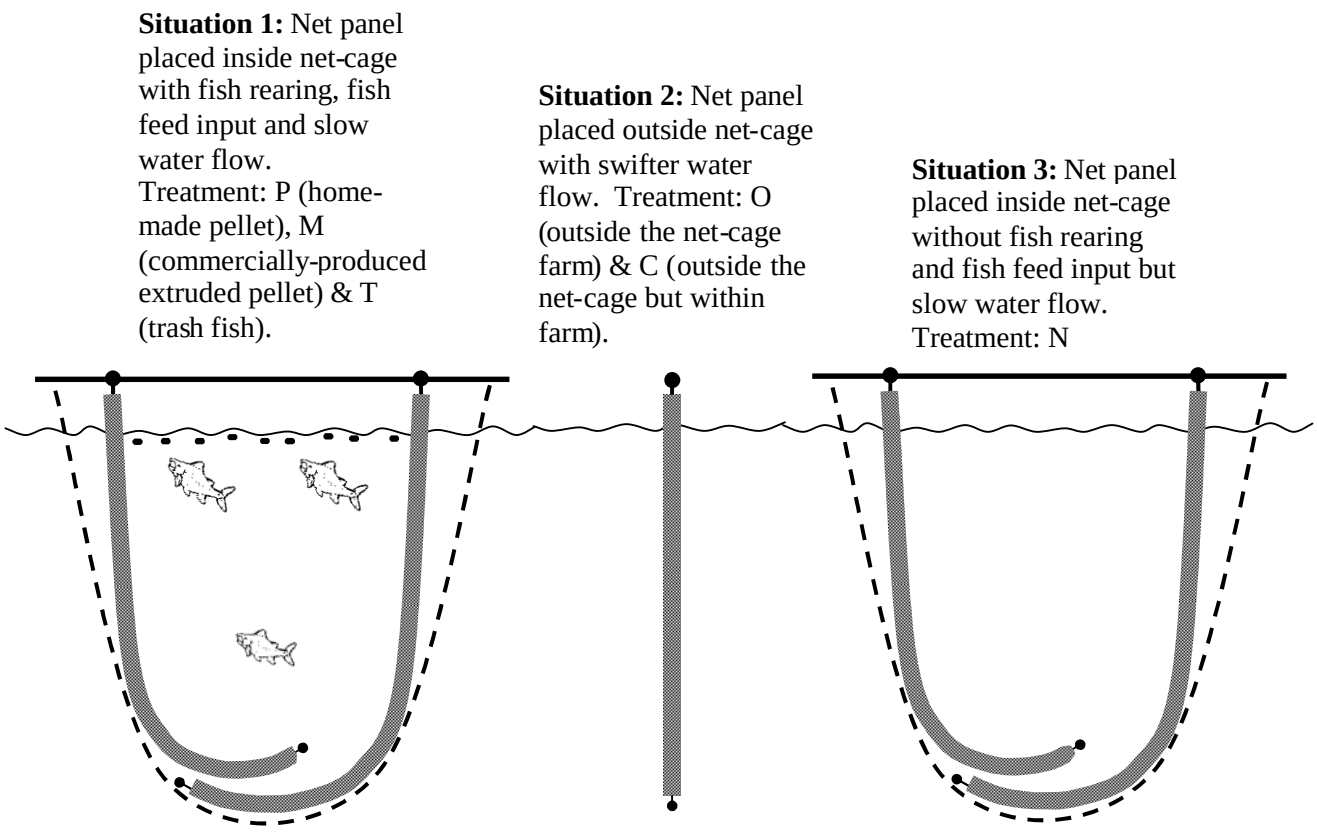


Figure 2.10. Sketch diagram showing 3 situations of testing used in this study. See Figure 2.3 for details.

CHAPTER 3

COMMUNITY STRUCTURE, SHORT-TERM COLONIZATION DYNAMICS AND BIOMASS OF MACROFOULING ASSEMBLAGES ON NETS

Summary of Important Findings

Eight species (7 phyla) of sessile macrofoulers and 27 species (3 phyla) of non-sessile organisms were recorded in a fish farm at Jaha and Sangga Besar River. Macrofouling assemblages began with colonization by hydroid *Plumularia* sp. irrespective of season, treatment (pellet 'P', trash-fish 'T', outside net-cage farm 'O') and estuary, while other species only appeared after 1 or 2 weeks of immersion. Inside net-cages given feed (whether trash-fish or pellet) where water flow was slow (mean < 6 cm s^{-1} at 0.50 – 0.75 m depth), macroalgae (*Polysiphonia* sp.), anthozoans (unidentified anemone), barnacles (*Balanus amphitrite*), amphipods (*Gammaropsis* sp. & *Photis* sp.) and tanaids (*Leptognathia* sp.) were dominant on net panels during the dry season. In the wet season, hydroid (*Plumulari*a sp.), mussel (*Xenostrobus mangle*) and nematode abundance increased significantly. With stronger water flow (mean ≈ 20 cm s^{-1}) and without feed input as occurring outside the net-cages, macrofouling assemblages for both seasons comprised mainly *Plumularia* sp. and *Gammaropsis* sp.. The macrofouling assemblage showed a clear succession of species that occupied different layers of the net panels. The study shows that while organic enrichment and retarded water flow together enhanced the development of macrofouling assemblages, salinity, depth, substrate (net) area and species competition specifically influenced community structure, colonization and depth distribution of the macrofouling organisms.

"Part of the content of this chapter was published in international conferences and ISI indexed journal as follows:

i) Madin, J. & Chong, V.C. (2004). Effect of fish feed on biofouling development in floating fish cages In: Marine Science Into the New Millennium; New Perspectives and Challenges, Proceedings of the Asia-Pacific Conference on Marine Sciences & Technology, 12–16 May

2002, Kuala Lumpur, Malaysia (ed. S.M. Phang, V.C. Chong, S.C. Ho, N. Mokhtar & L.S. Jillian Ooi), University of Malaya, Kuala Lumpur. pp. 307–324 (Appendix 1).

ii) John Madin, V.C. Chong & Badrulnizam Basri (2009). Development and short-term dynamics of macrofouling assemblages on fish-cage nettings in a tropical estuary. Estuarine, Coastal and Shelf Science 83 (2009) 19–29 (Appendix 2)*"*.

3.1. INTRODUCTION

Macrofouling assemblages on artificial structures have been extensively studied for a variety of purposes including the empirical models that are used for studying their succession, colonization, settlement, competition, recruitment (e.g. Mook, 1981; Greene et al., 1983; Oshurkov, 1992; Holmstrom & Kjelleberg, 1994; Abarzua & Jakubowski, 1995; Butler & Connolly, 1996; Anderson & Underwood, 1994; Jacobi & Langevin, 1996) and stability of assemblages (e.g. Kay & Butler, 1983; Butler & Connolly, 1996). Another important purpose is to study the practical problem of biofouling prevention on artificial substrata (e.g. Abarzua & Jakubowski, 1995).

The nature and abundance of biofouling organisms are influenced by geographical location, season, and such biotic factors as larval supply, predation and their ability to compete for the available attachment surface (Connell, 2001; Callow & Callow, 2002). The abiotic factors such as substratum type, temperature, salinity, current strength and water depth are also known to influence biofouling assemblages (Underwood & Keough, 2001; Witman & Dayton, 2001). Biofouling assemblages on artificial structures are characterized by continuous changes in species composition in response to the biotic and abiotic factors over time (Greene & Schoener, 1982).

Research on biofouling in the aquaculture industry have concentrated mainly on the methods of prevention, cleaning and control of biofouling by bacteria, diatoms, algae and invertebrates on artificial structures associated with the aquaculture operation (e.g. Milne & Powell, 1967; Dempsey, 1981a, b; Hodson & Burke, 1994; Hodson et al.,

1997; Hodson et al., 2000; Ross et al., 2004; Lodeiros & Garcia, 2004; Braithwaite et al., 2007; Corner et al., 2007). There is however, little knowledge on the community structure and dynamics of succession and colonization of biofouling organisms on artificial structures associated with aquaculture operation (e.g. Cook, 2001; Braithwaite et al., 2007). These included a couple of studies in temperate waters' for example, Hall (1995) studied the composition of macrofouling communities on aquaculture nets at three geographically distinct salmon farms in Canadian's Atlantic waters. Hodson et al. (2000) partly studied the development, composition, adhesion and preferential settlement of biofouling organisms in salmon cages in Autsralia, while Greene & Grizzle (2007) examined the ecological succession of biofouling communities on nettings of fish cages in the western Gulf of Maine, USA. In the tropical waters, information of macrofouling assemblages in aquaculture is confined to the work of Cheah & Chua (1979) who conducted a preliminary study of macrofouling composition on floating net-cages in Malaysia.

There are several factors thought to influence the macrofouling assemblages in aquaculture. Greene & Grizzle (2007) indicate that predation is a major factor affecting the development of biofouling communities on fish cages, while Hall (1995) determined that composition of macrofouling communities was variously influenced by the time of year, location, and depth. A number of biofouling-related studies in tropical fish farms have suggested that species composition and quantity reflect the condition of water quality (Huang et al., 1999) and the added nutrient associated with aquaculture operation (Ruokolahti, 1988), while the development rates are influenced by the mesh size of the net-cages used (Cheah & Chua, 1983).

The use of most commercially available, anti-fouling chemicals or coatings on cage nettings is largely restricted due to concern of environmental toxicity as well as consumer preference that may jeopardize the market image of cultured fish (see Wu,

1995; Champ, 2000; Braithwaite et al., 2007). For these reasons, it is imperative that the natural control of biofouling or environment-friendly methods be used. Such methods would require a good understanding of the fouling community of cage nettings and how it interacts with the physical (culture) environment. Thus, the present study was carried out in order to acquire a better understanding of where, when, how and why macrofouling assemblages develop on fish cage nettings. The experiment was carried out by studying the development of macrofouling assemblages on suspended experimental netting panels that are identical to the nettings of floating net-cages culturing fish in coastal embayments and estuaries.

The specific objectives of this study were to identify and quantify the macrofouling community composition, abundance and biomass, spatially (by depth) and temporally, and in relation to fish rearing and season. To investigate the effects of (factors associated with) fish rearing, the net panels were placed inside net-cages with reared fish (hence panels will be in an impeded water flow regime and exposed to uneaten/broken up fish feed, fish metabolites and faeces) and outside the net-cages (hence panels were in an unimpeded flow regime with none of the above stated inputs). Two types of fish feed were used; trash-fish, the normal feed type used in fish cage culture in Malaysia, and formulated home-made pellet feed which is considered less polluting. To test for the effects of season, the identical experiment was carried out in the "wet" and "dry" seasons of the year.

3.2. RESULTS

3.2.1. Environmental Conditions

The monthly rainfall regime from 1999 – 2002 indicates periods of heavier rainfall in between drier periods (Figure 3.1). Two such periods of heavier rainfall generally fell from April – May and November – December, coinciding with the onset of the

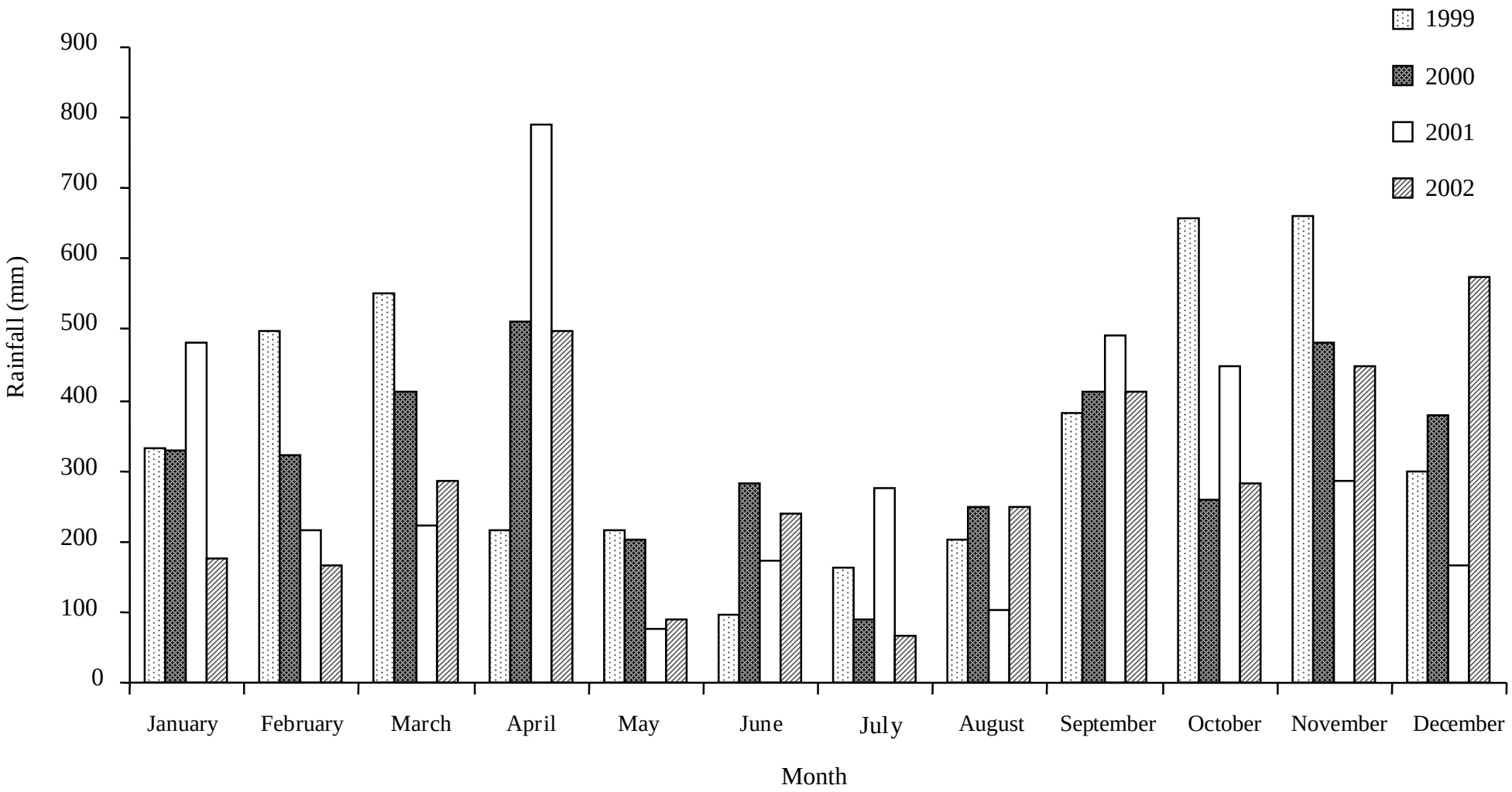


Figure 3.1. Monthly rainfall data at Taiping Hospital in the years 1999, 2000, 2001 and 2002 (Department of Meteorology, Taiping, Perak Malaysia).

Southwest and Northeast Monsoon periods respectively. Nevertheless, the monsoon periods experience periods of dry spells particularly in the months of July and August, and in February. Thus, for the purpose of this study, November was taken as the start of the "wet season" and July as the start of the "dry season" of the experiments.

The mean daily rainfalls during the wet season of the experiment were generally higher from November 2000 – January 2001 with an average 14.59 mm as compared to 5.50 mm from July – August 2000 for the dry season of the experiment (Figure 3.2). Except for the day 15th of August 2000 where the mean rainfall was exceptionally high at 61 mm, the other mean daily rainfalls were significantly ($P < 0.05$) much lower during the "dry season" experiment than in the "wet season" experiment.

The variations in some of the main physical parameters measured during the study are shown in (Table 3.1). Salinity and turbidity readings for both surface and bottom were significantly higher ($P < 0.001$) during the dry season compared to the wet season at Jaha but temperature, pH and dissolved oxygen were not significantly different ($P > 0.05$). Surface salinity during the dry season averaged 24.9 ppt in comparison to 16.0 ppt recorded during the wet season at Jaha. Water current velocities measured outside the net-cages (O) were significantly ($P < 0.001$) higher than inside the net-cages given pellet (P) or trash-fish (T) feed. However there were no significant ($P > 0.05$) difference in current velocity between net-cages given pellet and trash-fish feed in both Jaha and Sangga Besar, or between the dry and wet seasons at Jaha. For feed given net-cages, there was also no significant ($P > 0.05$) attenuation of current velocities among the replicates, i.e. from P1 to P3 or T1 to T3 (Figure 3.3). During the dry season, pH, temperature, dissolved oxygen and salinity were higher in Sangga Besar than in Jaha, but turbidity and water current velocities were relatively lower.

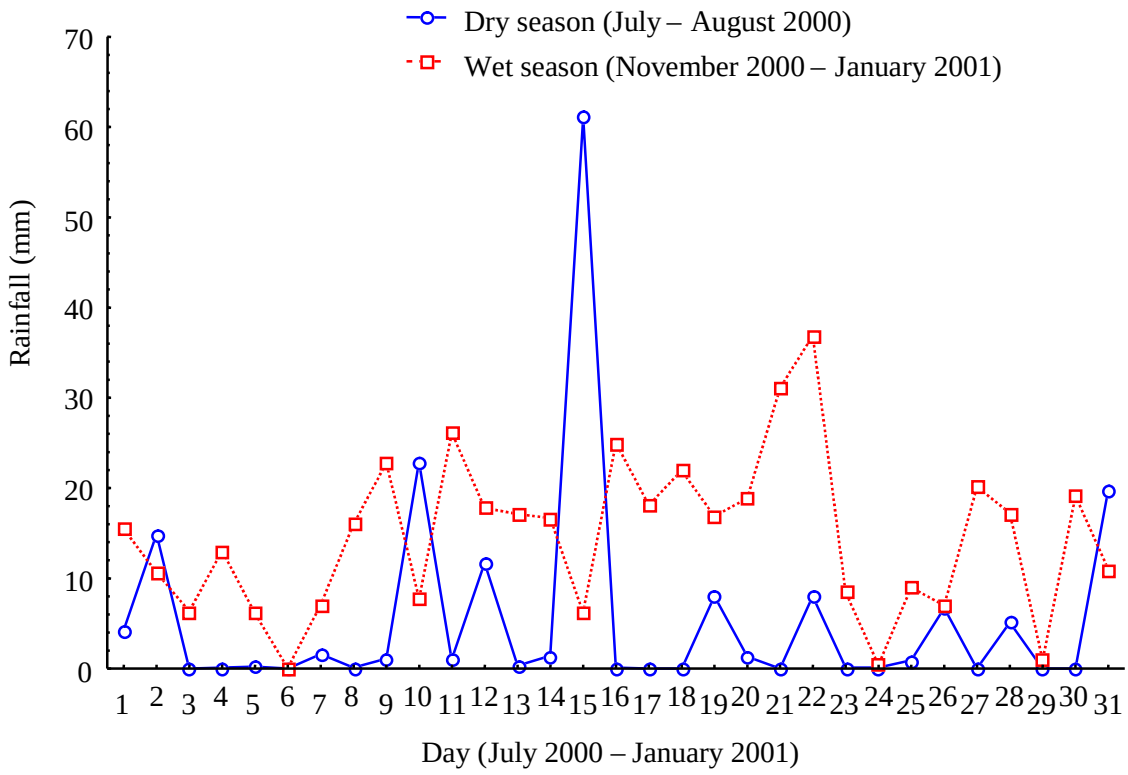


Figure 3.2. Mean daily rainfall during the study period (July 2000 – March 2001) at Taiping Hospital (Department of Meteorology, Taiping, Perak Malaysia).

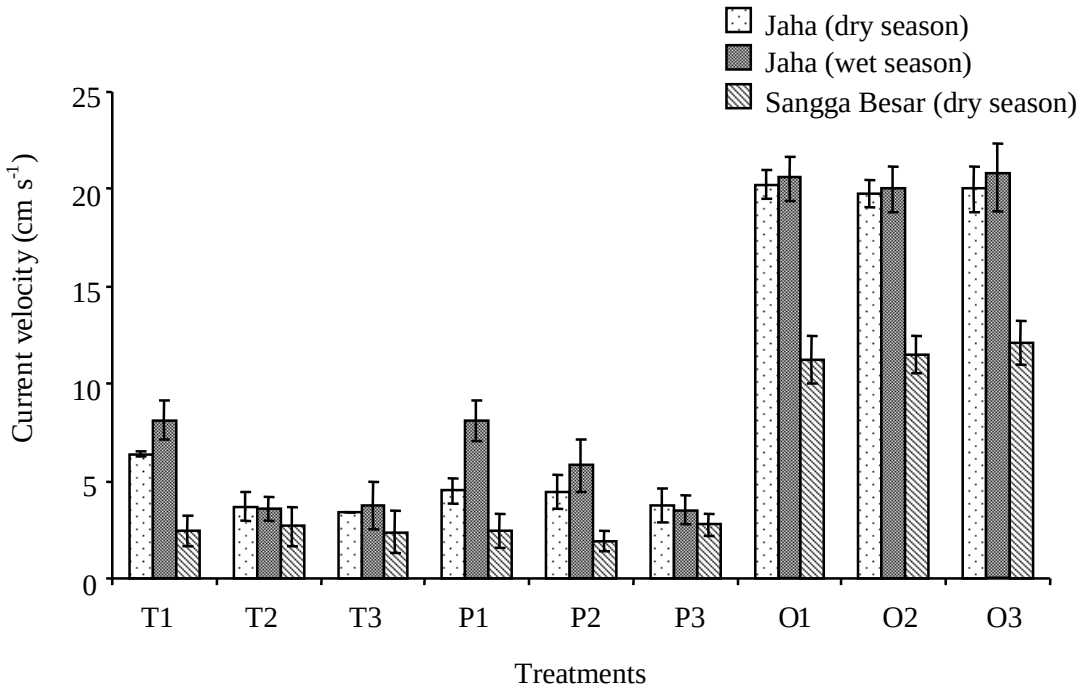


Figure 3.3. Mean water current velocity ± SD (cm s^{-1}) recorded at T1, T2 and T3 (inside the net-cages given trash-fish feed), P1, P2, and P3 (inside the net-cages given pellet feed), and O1, O2 and O3 (outside the net-cages, no fish and feed) during the dry and wet season in a fish farm at Jaha and the dry season only at Sangga Besar.

Table 3.1. Mean values of some environmental parameters recorded inside net-cages given trash-fish feed (T), pellet feed (P) and no feed outside the net-cages (O) in a fish farm at (a) Jaha (dry season), (b) Jaha (wet season) and (c) Sangga Besar (dry season). Standard deviation (SD) in parentheses.

a. Jaha (dry season)

Parameter	Trash-fish feed (T)		Pellet feed (P)		Outside the net-cages (O)	
	surface	bottom	surface	bottom	surface	bottom
pH	7.08 (0.11)	6.90 (0.13)	7.04 (0.12)	6.82 (0.11)	7.05 (0.11)	6.93 (0.15)
Temperature (ºC)	30.00 (1.38)	29.80 (1.30)	29.67 (1.39)	29.68 (1.44)	29.62 (1.31)	29.95 (1.54)
Salinity (ppt)	24.87 (4.35)*	24.01 (4.27)*	24.84 (4.49)*	24.13 (4.36)*	25.07 (4.49)*	24.48 (4.19)*
DO (mg l^{-1})	3.73 (1.99)	3.27 (1.19)	3.74 (1.97)	3.07 (1.48)	3.63 (1.97)	3.18 (1.63)
Turbidity (NTU)	25.33 (6.02)*	34.87 (12.38)*	13.80 (5.68)*	21.00 (7.74)*	12.67 (7.34)*	46.60 (6.00)*
Water velocity (cm s^{-1})	4.47 (3.87)		3.80 (1.00)		20.00 (1.60) †	

b. Jaha (wet season)

pH	7.10 (0.54)	6.88 (0.40)	7.00 (0.51)	6.86 (0.44)	6.97 (0.53)	6.88 (0.53)
Temperature (ºC)	29.42 (1.01)	30.25 (1.05)	29.35 (0.93)	30.22 (0.91)	29.42 (0.89)	30.33 (0.94)
Salinity (ppt)	15.57 (6.20)	18.22 (6.20)	15.83 (5.99)	18.47 (6.22)	16.72 (6.59)	18.15 (7.01)
DO (mg l^{-1})	4.44 (2.73)	3.43 (2.71)	4.65 (2.66)	3.05 (2.50)	4.14 (2.66)	2.75 (2.36)
Turbidity (NTU)	4.52 (1.50)	5.14 (1.90)	5.57 (3.03)	5.29 (1.35)	5.48 (1.63)	7.90 (2.47)
Water velocity (cm s^{-1})	5.14 (4.47)		5.80 (5.00)		20.80 (4.80) †	

c. Sangga Besar (dry season)

pH	7.25 (0.19)	7.17 (0.16)	7.19 (0.20)	7.14 (0.19)	7.17 (0.17)	7.13 (0.16)
Temperature (ºC)	30.15 (0.45)	30.70 (0.50)	30.08 (0.34)	30.61 (0.55)	29.77 (0.43)	30.62 (0.41)
Salinity (ppt)	28.31 (3.14)	28.77 (2.91)	28.18 (2.94)	28.78 (2.91)	28.03 (3.22)	28.82 (3.01)
DO (mg l^{-1})	4.05 (1.59)	3.65 (1.69)	4.06 (1.42)	3.23 (1.07)	4.09 (1.52)	3.21 (0.80)
Turbidity (NTU)	5.4 (0.73)	9 (1.73)	16.93 (5.87)	21.46 (5.33)	15.6 (6.11)	21.06 (5.62)
Water velocity (cm s^{-1})	2.89 (1.68)		2.72 (1.39)		14.67 (6.53) †	

† indicates significant different ($P < 0.05$) amongst treatment (T, P, O)
* indicates significant different ($P < 0.05$) between dry and wet seasons in Jaha

3.2.2. Overview of Macrofouling Dynamics in Relation to Fish Rearing and Season at Jaha as Revealed by PCA

The first two axes derived from PCA of the species abundance data explained 42% of the total variance in the species abundance data. The factor loadings or eigen vectors show that the first axis or PC1 is primarily a descriptor of the hydroid *Plumularia* sp. (Plu), its abundance gradient in the positive direction, while describing the abundance of *Photis s*p. (Pho), *Leptognathia* sp. (Lep), *Balanus amphitrite* (Bal), *Polysiphonia* sp. (Pol) and unidentified anemones (Ant) in the negative direction. PC2 is a descriptor of the abundance of particularly *Euterpina acutifrons* (Eut), *Xenostrobus mangle* (Xen) and nematode worms (Nem) in the negative direction. Interpretation of the PCA results is better illustrated in (Figure 3.4), which shows the biplots of sites or net panels (symbols □, O, Δ) and macrofaunal species (arrows).

The PCA biplots illustrate two courses of net colonization by sessile macrofoulers inside the net-cages, based on the community structure which was determined by season and period of immersion (top and bottom left quadrants). For both seasons, the earliest colonizer at all depths was the colonial hydroid, *Plumularia* sp. However, in the dry season, as colonization proceeded to the 3rd or 4th week, the hydroid was replaced by the macroalgae *Polysiphonia* sp. and *Enteromorpha clathrata*, as well as by anthozoans (unidentified anemone) and *Balanus amphitrite.* On the other hand, the biofouling development in the wet season appeared much slower, and the hydroid were displaced by yet another, increasingly dominant mussel species, *Xenostrobus mangle*, after 6 weeks of submersion.

Inside the net-cages, the rate of colonization appeared to be more affected by feed input (with or without feed) rather than by the type of feed given (pellet or trash-fish feed). Outside the net-cages (no feed), the type of net macrofouling species and their abundance did not differ significantly with season, or the period of immersion (top right

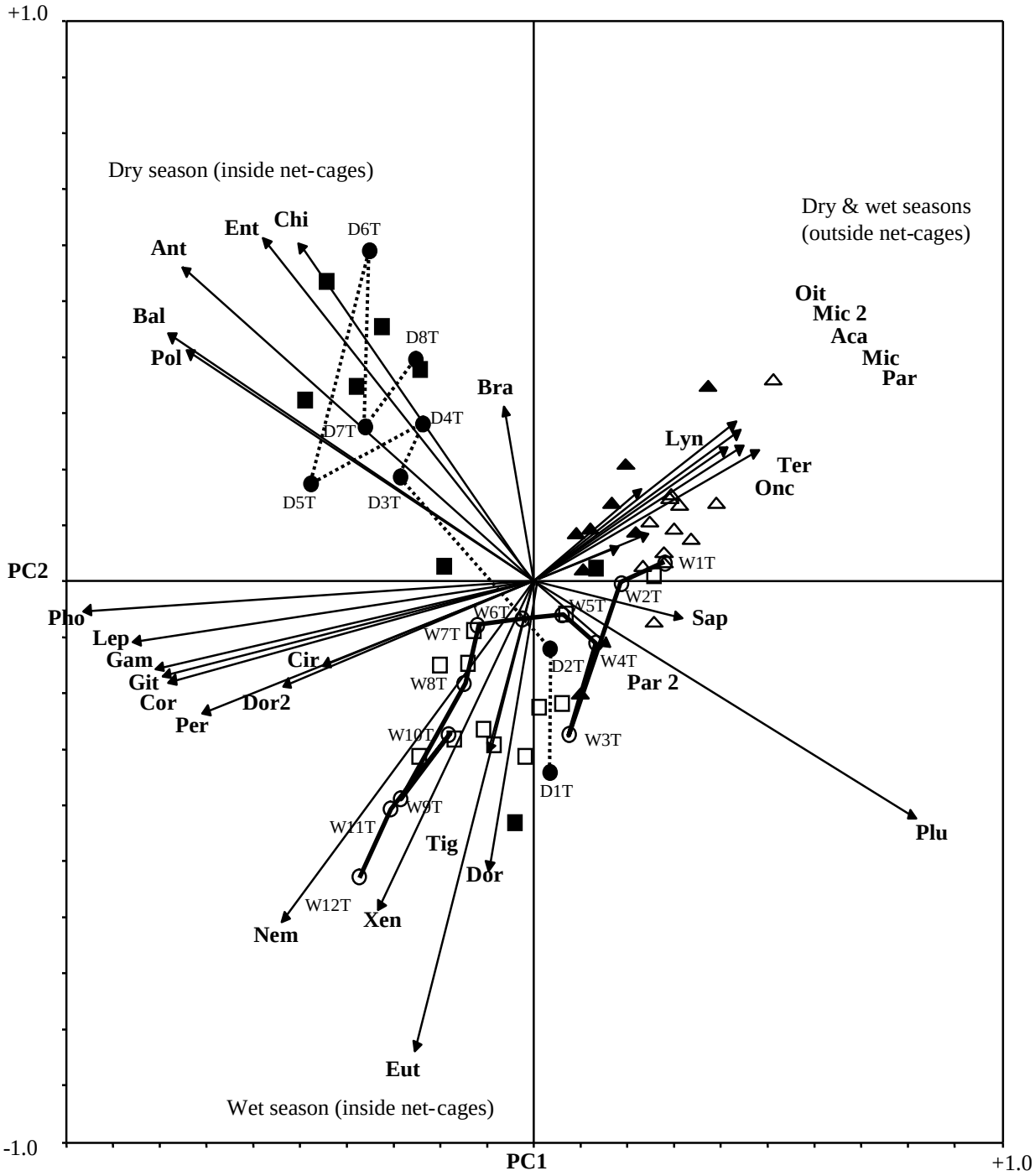


Figure 3.4. PCA biplots of the abundance of net panel macrofouling organisms (arrows) on experimental net-panels (squares, circles and triangles) for dry and wet seasons. Solid and dash lines linking filled or open circles trace the weekly (numerals) macrofouling progression in net-cages given trash-fish feed (T) during wet (W) and dry (D) seasons respectively. Types of treatment: pellet feed in dry season (■); pellet feed in wet season (□); trash-fish feed in dry season (●); trash-fish feed in wet season (○); outside net-cages in dry season (▲); outside net-cages in wet season (Δ). Full taxa names are given in Table 3.2.

quadrant). These macrofoulers comprised of mainly the same hydroids and associated non-sessile organisms, copepods.

Among the non-sessile organisms that were closely associated with the sessile macrofoulers inside the fish cages were several amphipod species. These amphipods together with a few other species form a non-sessile assemblage that was observed in both the dry and wet seasons (see Figure 3.4). Only *Cheirophotis* sp. appeared to be associated with the dry season. *Gammaropsis* sp. was also more abundant during the dry season. In trash-fish cages, it dominated for the first 4 weeks but dropped thereafter. Another amphipod species, *Photis* sp. gradually increased in numbers, exceeding *Gammaropsis* sp. by the 5th week. In the pellet-given cages, a maximum density achieved by *Gammaropsis* sp. obtained during the first two weeks, but similarly decreased at the 5th week, only to be exceeded by *Photis* sp.. Outside the net-cages, the abundance of *Gammaropsis* sp. was always higher than that of *Photis* sp.. Generally, the populations of other associated non-sessile organisms, such as copepods, polychaetes, tanaids and nematodes, increased rapidly up to the 2nd week, but their numbers slightly decreased and stabilized thereafter.

In the wet season, the associated non-sessile organisms were the harpacticoid copepod, *Euterpina acutifrons*, and nematode worm which were not characteristically abundant during the dry season. During the wet season, the numbers of *Gammaropsis* sp. in the feed-receiving cages initially increased rapidly but somewhat slowed down from the 6th week onward, only to be exceeded by *Photis* sp. by the 9th week. Other amphipods were present in relatively smaller numbers, but as in the dry season, their numbers in fish rearing cages were still much higher than outside the net-cages. Populations of other non-sessile macrofoulers were very variable during the first 6 weeks of submersion, and their numbers gradually increased after the 7th week (detailed data appended in Appendix 3).

3.2.3. Sessile Macrofouling Organisms of Net Panels Suspended Inside Net-Cages (Trash-Fish Feed, Dry Season)

3.2.3.1. Species Composition

During the dry season, sessile macrofouling community on net panels placed inside the net-cages given trash-fish feed comprised of 8 species from 7 phyla namely Rhodophyta (*Polysiphonia* sp.), Chlorophyta (*Enteromorpha clathrata),* Cyanophyta (*Lyngbya* sp.), Mollusca (*Xenostrobus mangle*), Cnidaria (unidentified anemone, *Plumularia* sp.), Arthropoda (*Balanus amphitrite*) and Bryozoa *(Cryptosula* sp.) (Table 3.2) (Plate 3.1). Almost all sessile macrofouling species were similar for Jaha and Sangga Besar except for bryozoans *(Cryptosula* sp.) which were only encountered in Sangga Besar. The organic or inorganic materials present on net panels consisted of unidentified detritus and tube burrows of non-sessile species.

3.2.3.2. Depth Distribution and Percentage Cover

Algal fouling of *Polysiphonia* sp. and *E. clathrata* dominated the upper stratum of net panels placed inside the net-cages given trash-fish feed at Jaha. *Plumularia* sp., anthozoans (unidentified anemone), *B. amphitrite, Lyngbya* sp. and *X. mangle* were not depth preferential but intense competition may have caused their populations to be concentrated at particular depths. *Polysiphonia* sp. occupied the upper stratum with mean cover of 57.2%, while covers of *E. clathrata*, *Plumularia* sp. and anthozoans were 16%, 13.2% and 8.3% respectively (Figure 3.5a).

The covers of *Polysiphonia* sp. and *E. clathrata* were reduced drastically at the middle and bottom strata due to higher percentage cover of *Plumularia* sp. and anthozoans. Mean percentage cover of *Plumularia* sp. was 42.6% and 54.9% at the middle and bottom stratum respectively, while it was 32.6% and 32.7% respectively for anthozoans. *B. amphitrite* cover was 11% and 10.6% respectively at the middle and

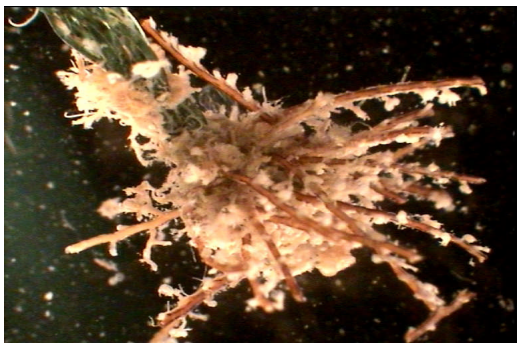
Table 3.2. List of sessile and non-sessile macrofouling organisms found in a fish farm at Jaha and Sangga Besar. Acronyms are given in parentheses.

Sessile Organisms

Division Rhodophyta
Class Rhodophyceae
Order Gigartinales
Family Rhodomelaceae
Polysiphonia sp. *(*Pol)
Division Chlorophyta
Class Chlorophyceae
Order Ulitrichales
Family Ulvaceae
Enteromorpha clathrata (Ent)
Division Cyanophyta
Class Cynophyceae
Order Nostocales
Family Oscillatoriaceae
Lyngbya sp. (Lyn)
Phylum Mollusca
Class Bivalvia
Subclass Pteriomorphia
Order Mytiloida
Family Mytilidae
Xenostrobus mangle (Xen)
Phylum Cnidaria
Class Anthozoa
Subclass Hexacorallia
Order Actiniaria
Unidentified sea anemone (Ant)
Class Hydrozoa
Order Hydroida
Suborder Leptomedusae
Family Plumulariidae
Plumularia sp. (Plu)
Phylum Arthropoda
Subphylum Crustacea
Class Maxillopoda
Subclass Thecostraca
Order Sessilia
Family Balanidae
Balanus amphitrite (Bal)
Phylum Bryozoan
Family Cheilostomata
Cryptosula sp.

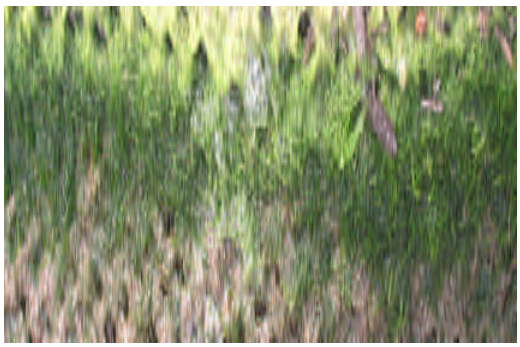
Non-sessile Organisms

Phylum Arthropoda
Subphylum Crustacea
Class Malacostraca
Order Amphipoda
Suboder Gammaridea
Family Isaeidae
Gammaropsis sp. (Gam)
Photis sp. (Pho)
Cheirophotis sp. (Chi)
Family Corophiidae
Corophium sp. (Cor)
Family Amphilochida
Gitanopsis sp. (Git)
Order Tanaidacea
Suborder Tanaidomorpha
Family Leptognathiidae
Leptognathia sp. (Lep)
Order Isopoda
Suborder Flabellifera
Family Cirolanidae
Cirolana sp. (Cir)
Order Decapoda
Brachyura megalopa (Bra)
Class Maxillopoda
Subclass Copepoda
Order Harpaticoida
Family Euterpinidae
Euterpina acutifrons (Eut)
Family Harpacticidae
Tigriopus sp. (Tig)
Order Poecilostomatoida
Family Oncaeidae
Oncaea sp. (Onc)
Family Sapphirinidae
Saphierella-like copepodid (Sap)
Order Calanoida
Family Acartiidae
Acartia pasifica (Aca)
Family Clausocalanidae
Microcalanus sp. 1 (Mic)
Microcalanus sp. 2 (Mic 2)
Family Paracalanidae
Paracalanus sp. 1 (Par)
Paracalanus sp. 2 (Par 2)
Order Cyclopoida
Family Oithonidae
Oithona simplex (Oit)
Unidentified copepod larvae
Order Sessilia
Family Balanidae
Unidentified Balanoid larvae
Phylum Annelida
Class Polychaeta
Subclass Palpata
Order Aciculata
Suborder Phyllodocida
Family Nereididae
Perinereis sp. (Per)
Suborder Eunicida
Family Dorvilleidae
Dorvilleidae sp. 1 (Dor)
Dorvilleidae sp. 2 (Dor 2)
Order Canalipalpata
Suborder Terebellida
Family Terebellidae
Terebellidae sp. (Ter)
Unidentified Polychaete juvenile
Phylum Nematoda
Undetermined species (Nem)
Phylum Mollusca
Class Bivalvia
Unidentified Bivalve veliger



(a)

(b)



(c)

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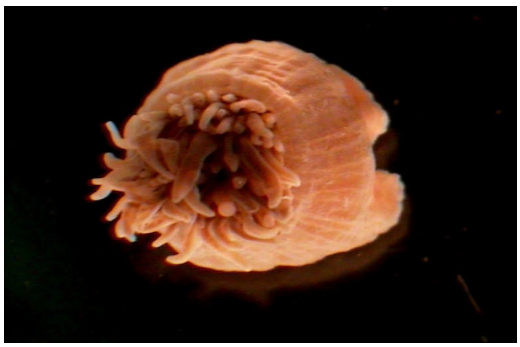

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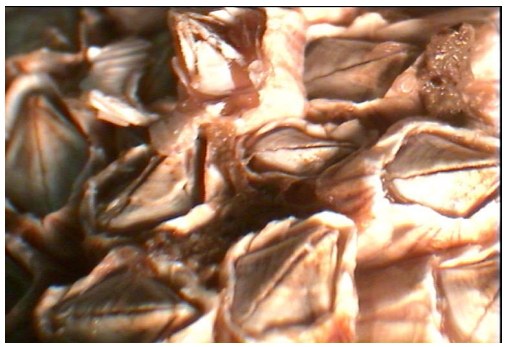

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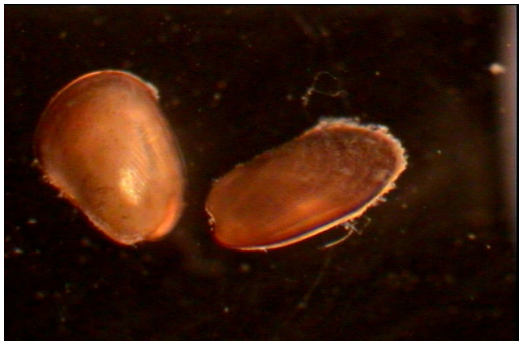
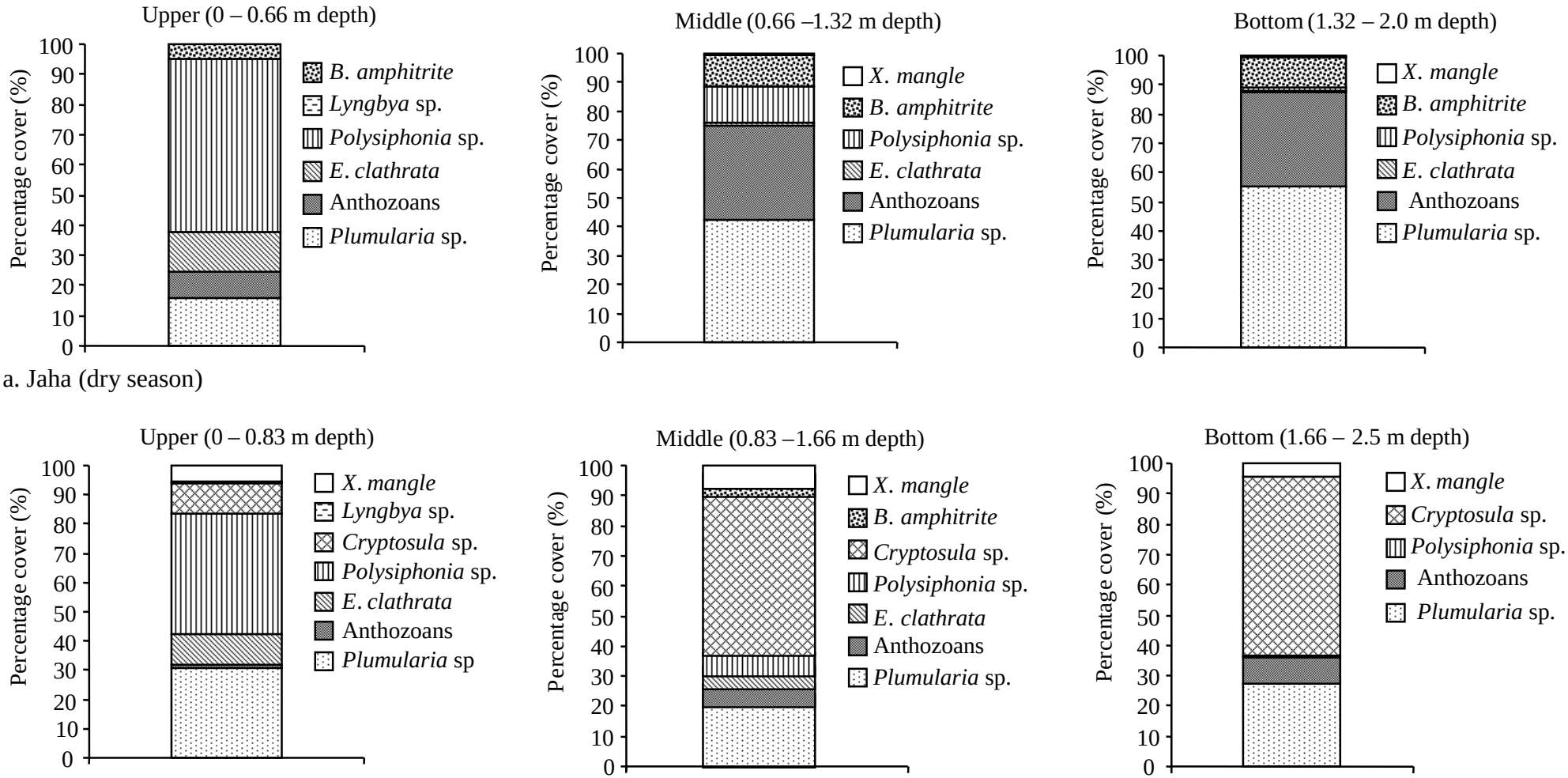


(g)

(h)

Plate 3.1. Sessile macrofouling species found in a fish farm at Jaha and Sangga Besar River including (a) *Plumularia* sp., (b) *Polysiphonia* sp., (c) *Enteromorpha clathrata*, (d) *Lyngbya* sp., (e) Anthozoans (unidentified anemone), (f) *Balanus amphitrite*, (g) X*enostrobus mangle* and (h) *Cryptosula* sp..



a. Jaha (dry season)

b. Sangga Besar (dry season)

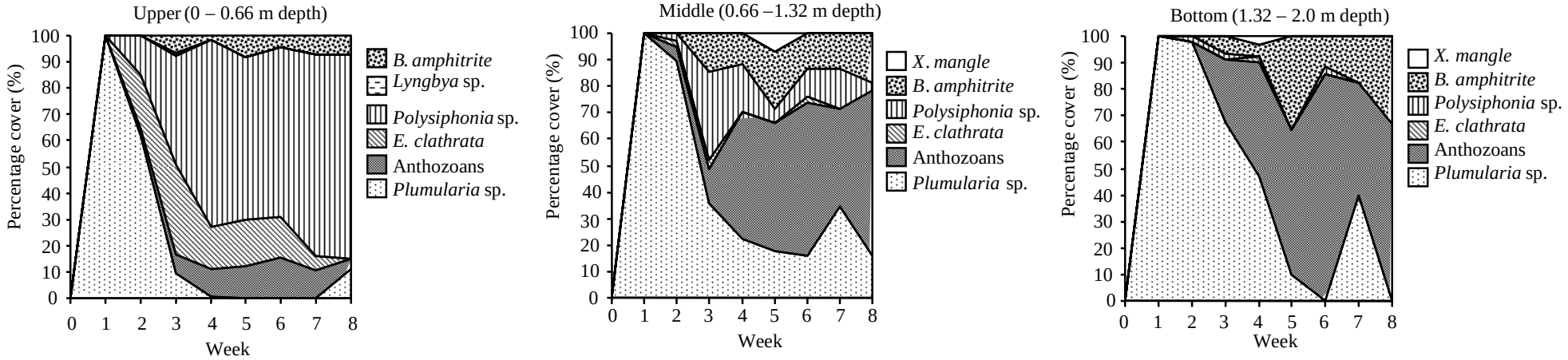
Figure 3.5. Depth distribution (upper, middle & bottom) and percentage cover of sessile macrofouling organisms over eight weeks of colonization on net panels placed inside the net-cages given trash-fish feed in a fish farm at (a) Jaha (dry season) and (b) Sangga Besar (dry season).

bottom stratum while *X. mangle* and *Lyngbya* sp. were present with very small cover (< 1%) (detailed data appended in Appendix 4).

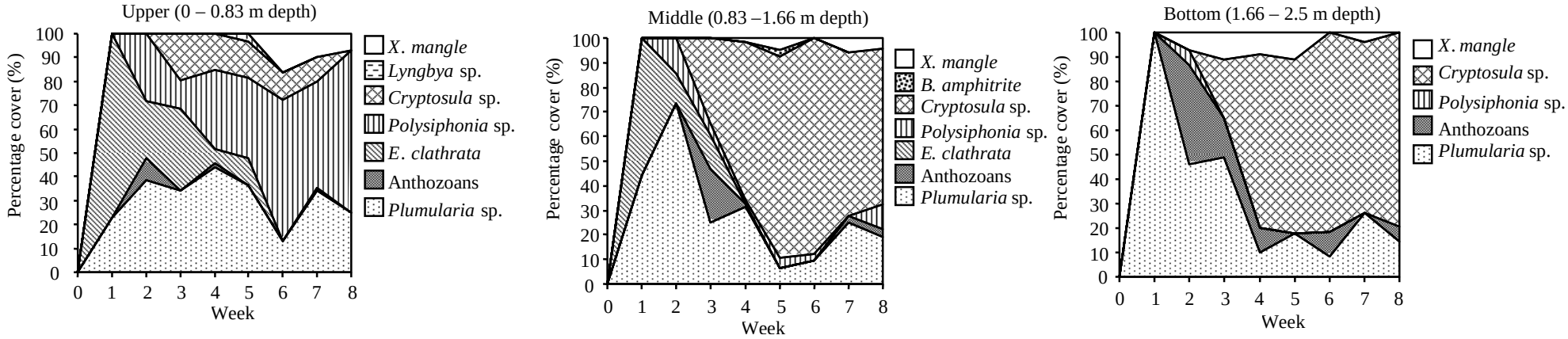
Similar to the results obtained from Jaha, the upper stratum of net panels placed inside the net-cages given trash-fish feed at Sangga Besar were dominated by *Polysiphonia* sp. with mean cover of 40.9% (Figure 3.5b). *Plumularia* sp. was another important fouler at the upper stratum with 31% cover, followed by *E. clathrata* and *Cryptosula* sp. with 10.8% and 10.3% cover respectively. Percentage cover of other species was less than 5% cover. Unlike the result in Jaha, percentage covers of *Plumularia* sp. and anthozoans at the middle and bottom stratum in Sangga Besar were reduced due to higher percentage cover of *Cryptosula* sp.. *Cryptosula* sp. dominated the middle and bottom stratum with mean covers of 59.4% and 58.8% respectively. The percentage cover of *B. amphitrite, Lyngbya* sp. and *X. mangle* were much lower (i.e. <10%). In both Jaha and Sangga Besar, the accumulation of organic and/or inorganic matter was relatively higher at the middle and bottom strata.

3.2.3.3. Temporal Change in Species Composition and Percentage Cover

Plumularia sp. was an important early colonizer of net panels placed inside the net-cages given trash-fish feed. In Jaha, many species started to appear only after the first or second week of net panel immersion. However, *Plumularia* sp. rapidly developed within first week with the highest percentage cover at all depth strata. Its percentage cover was gradually reduced in the following weeks due to the vigorous growth of *Polysiphonia* sp. and *E. clathrata* at the upper stratum and anthozoans and *B. amphitrite* at the middle and bottom stratum (Figure 3.6a). On the 3rd week, the percentage cover of *Plumularia* sp. at the upper stratum was reduced rapidly to 9.5% while *Polysiphonia* sp. and *E. clathrata* increased to 41.6% and 33.8% respectively. *Polysiphonia* sp. continued to overgrow other species and achieved a maximum cover of 77.8% on the 8th week.



a. Jaha (dry season)



b. Sangga Besar (dry season)

Figure 3.6. Temporal changes in species composition and percentage cover of sessile macrofouling organisms at the upper, middle and bottom strata of net panels placed inside the net-cages given trash-fish feed in a fish farm at (a) Jaha (dry season) and (b) Sangga Besar (dry season).

Percentage cover of *Plumularia* sp. at the middle stratum was reduced drastically to 22.1% at 4th week due to increasing population of anthozoans (48%), *Polysiphonia* sp. (18.3%) and *B. amphitrite* (11.5%) (see Figure 3.6a). Anthozoans and *B. amphitrite* rapidly developed on the middle stratum from 2nd and 3rd week respectively, and achieved their maximum cover of 62.5% (anthozoans) and 18.8% (*B. amphitrite*) at 8th week. The cover of anthozoans was consistently higher at the middle and bottom stratum suppressing other competitors such as *Plumularia* sp., *Polysiphonia* sp. and *B. amphitrite.*

E. clathrata was among the earliest colonizers of the upper and middle strata of net panels placed inside the net-cages given trash-fish feed at Sangga Besar (Figure 3.6b). Its percentage cover at the upper stratum was 77.2% on the 1st week but reduced on the following week due to growing population of *Polysiphonia* sp., *Plumularia* sp. and *Cryptosula* sp.. On the 4th week, percentage cover of *E. clathrata* was reduced to 5.8% when *Plumularia* sp. and *Polysiphonia* sp. increased to 43.8% and 33.5% respectively. *Polysiphonia* sp. at the upper stratum started to develop on 2nd week and continually exceeded other species with a maximum cover of 67.7% at the 8th week.

Plumularia sp. was among the earliest colonizers in Sangga Besar, however percentage cover gradually reduced due to higher development rates of *Polysiphonia* sp. at the upper stratum, and *Cryptosula* sp. at the middle and bottom stratum respectively (see Figure 3.6b). *Cryptosula* sp. appeared on the week 2nd or 3rd, percentage cover increased rapidly to a maximum of 81.7% at the 5th week for middle and 81.7% at the 6th week for bottom stratum respectively. Anthozoans were present at all depths strata, a maximum cover of 40.6% at the bottom stratum achieved on the 2nd week. However, it gradually become reduced due to increasing population of *Cryptosula* sp.. Small numbers of *X. mangle*, *Lyngbya* sp. and *B. amphitrite* were occasionally present.

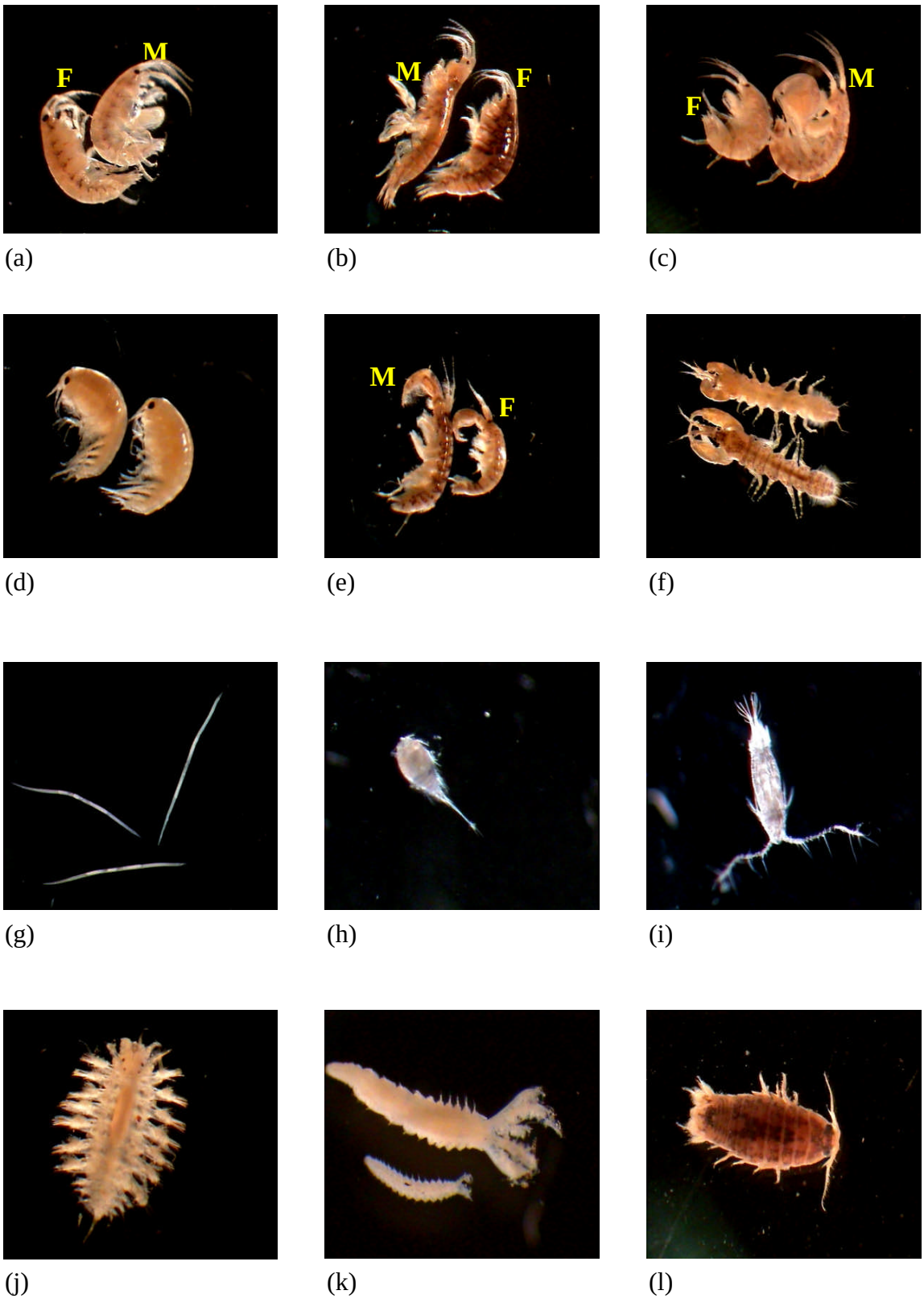
3.2.4. Non-Sessile Associates of Net Panels Suspended Inside Net-Cages (Trash-Fish Feed, Dry Season)

3.2.4.1. Species Composition

In Jaha, there were 22 species of non-sessile organisms belonging to 3 phyla, namely Arthropoda, Annelida and Nematoda, recorded on net panels placed inside the net-cages given trash-fish feed (see Table 3.2). The Arthropoda was represented by Amphipoda, Tanaidacea and Copepoda while Annelida and Nematoda were represented by polychaetes and an unidentified nematode respectively. Amphipoda was the major taxa of the non-sessile organisms which comprised five species, *Gammaropsis* sp., *Photis* sp., *Cheirophotis* sp., *Gitanopsis* sp. and *Corophium* sp. (Plate 3.2). The density of both *Gammaropsis* sp. and *Photis* sp. formed nearly 90% of the total non-sessile organisms on net panels. Tanaids and nematodes were among other important non-sessile organisms represented by a single species each, namely *Leptognathia* sp. and an undetermined species of nematode worm respectively.

Copepoda was the most diverse group with 6 species belonging to 4 orders including Harpacticoida (*Euterpina acutifrons* and *Tigriopus* sp.), Poecilostomatoida (*Oncaea* sp.), Calanoida (*Paracalanus* sp. 2 and *Acartia pasifica)* and Cyclopoida (*Oithona simplex*). Unidentified copepod larvae were invariably present on net panels. The copepods were dominated by *Euterpina acutifrons* while the densities of other species were relatively low or present occasionally. The class Polychaeta was represented by 4 species and unidentified juveniles but their densities were generally low as compared to amphipods, tanaids and copepods. Decapods, isopods and larvae of balanoids and bivalves (veliger) were occasionally encountered.

In Sangga Besar, a total of 19 non-sessile species were encountered on net panels placed inside the net-cages given trash-fish feed. The species composition was relatively similar for Jaha and Sangga Besar except for some minor species which occurred in



(a) (b) (c)

(d) (e) (f)

(g) (h) (i)

(j) (k) (l)

Plate 3.2. Non-sessile macrofouling species found in a fish farm at Jaha and Sangga Besar River including (a) *Gammaropsis* sp. (M-male & F-female), (b) *Photis* sp. (M-male & F-female), (c) *Cheirophotis* sp. (M-male and F-female), (d) *Gitanopsis* sp., (e) *Corophium* sp., (f) *Leptognathia* sp., (g) Undetermined nematode species, (h) *Euterpina acutifrons*, (i) *Acartia pasifica*, (j) Polycheate larvae, (k) *Terebellidae* sp. and (l) *Cirolana* sp.

very small numbers or occasionally encountered. *Oncaea* sp., *Oithona simplex*, *Acartia pasifica*, Saphierella-like copepodid, *Cirolana* sp. and brachyuran megalopa were not encountered in Sangga Besar.

3.2.4.2. Temporal Changes in Species Composition and Abundance

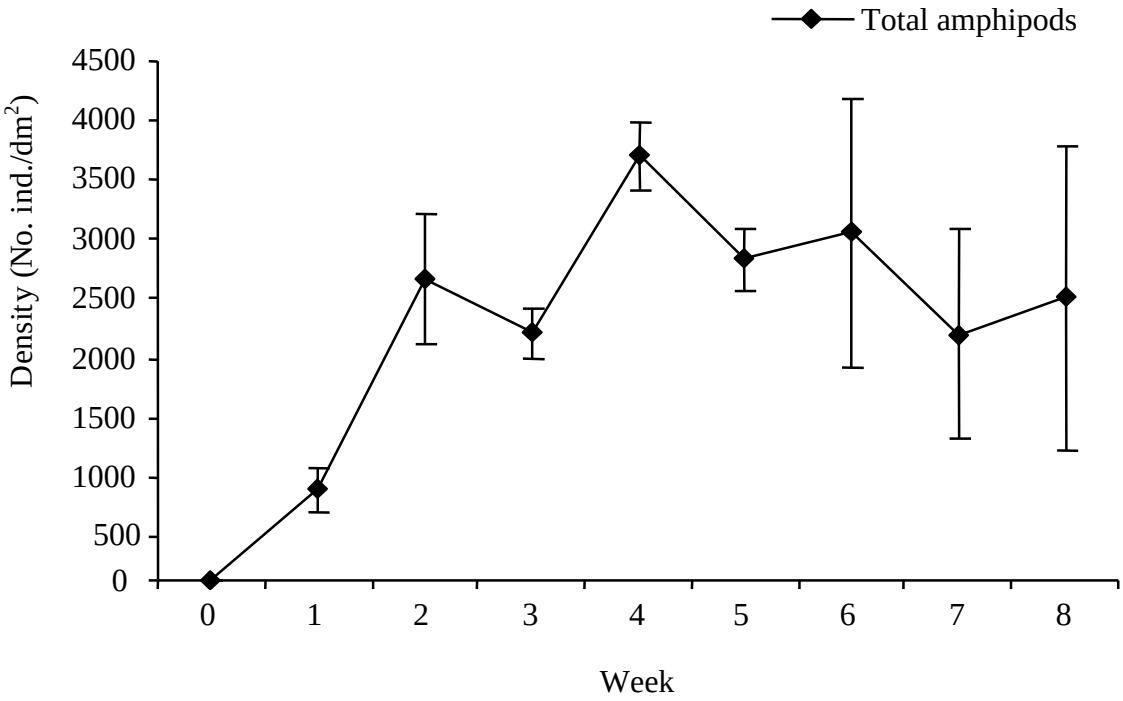
The non-sessile organisms are further elaborated according to taxa as follows:

a. Amphipoda

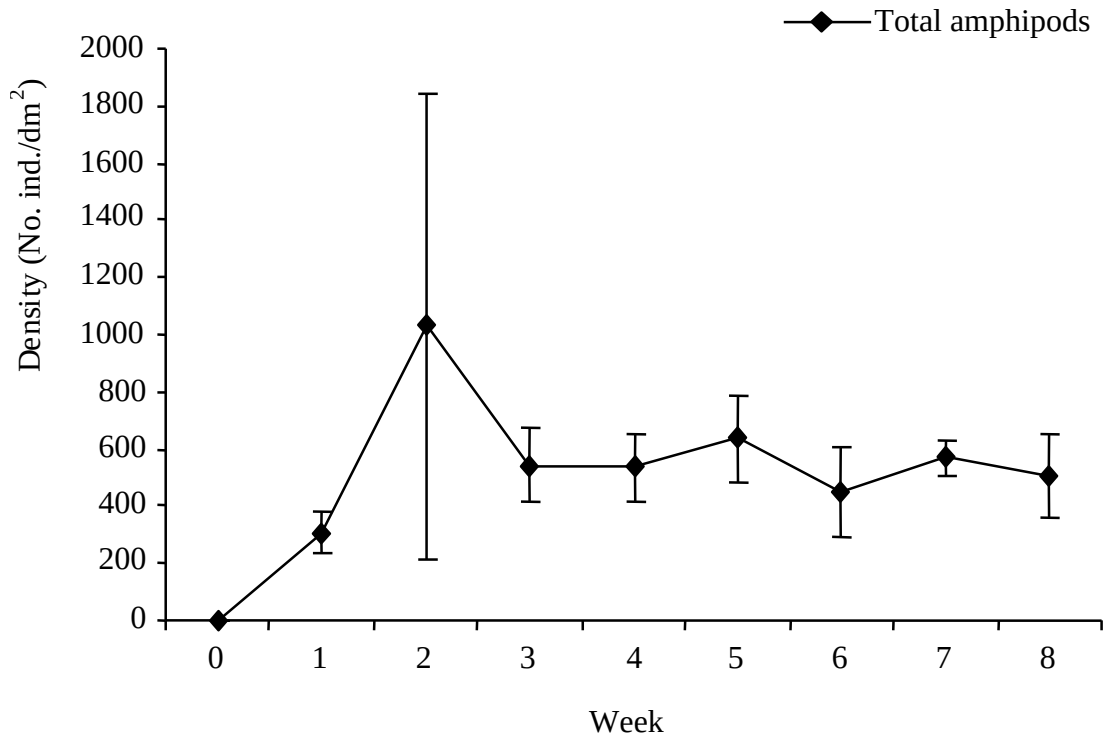
The amphipods were an important non-sessile macrofoulers of net panels placed inside the net-cages given trash-fish feed. The mean total density over eight weeks of colonization was 2,348.38 ind./dm^2 and 571.78 ind./dm^2 respectively at Jaha and Sangga Besar. The relatively lower density at Sangga Besar was probably due to the poor maintenance of net-cages after an outbreak of fish disease that caused mortality of cultured fish. In Jaha, weekly development rates of amphipods population were relatively higher, a maximum of 3,571.75 ind./dm^2 obtained on the 4th week (Figure 3.7a). In Sangga Besar, development rates were much slower than in Jaha, rarely exceeding 1000 ind./dm^2 (Figure 3.7b) (detailed data appended in Appendix 5).

Gammaropsis sp. and *Photis* sp. were the dominant amphipods species on net panels placed inside the net-cages given trash-fish feed in both Jaha and Sangga Besar. In Jaha, mean density of *Gammaropsis* sp. and *Photis* sp. over eight weeks of colonization was 1,490.33 ind./dm^2 and 840.58 ind./dm^2 respectively (Table 3.3). *Gammaropsis* sp. was the early colonizer, a maximum density of 3,135.73 ind./dm^2 obtained at the 4th week (Figure 3.8a). Density of *Gammaropsis* sp. dropped quickly to 930.34 ind./dm^2 on the 5th week due to increasing population of *Photis* sp. (1,745.03 ind./dm^2). The maximum density (1,762.07 ind./dm^2) of *Photis* sp. achieved at the 8th week, while *Gammaropsis* sp. had reduced to 595.31 ind./dm^2 (see Figure 3.8a).

In Sangga Besar, mean density of *Gammaropsis* sp. and *Photis* sp. over eight weeks



(a) Jaha (dry season)

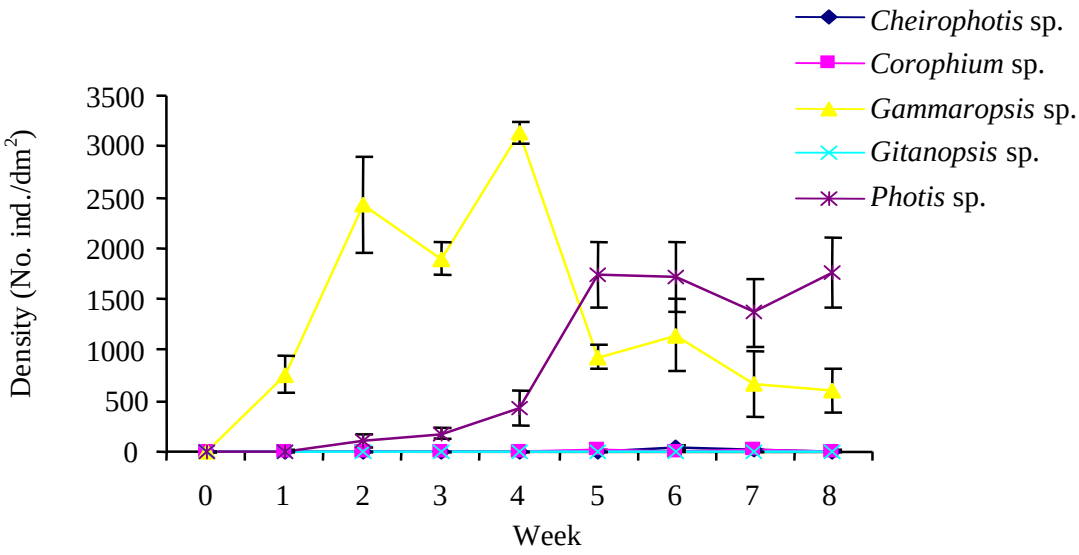


(b) Sangga Besar (dry season)

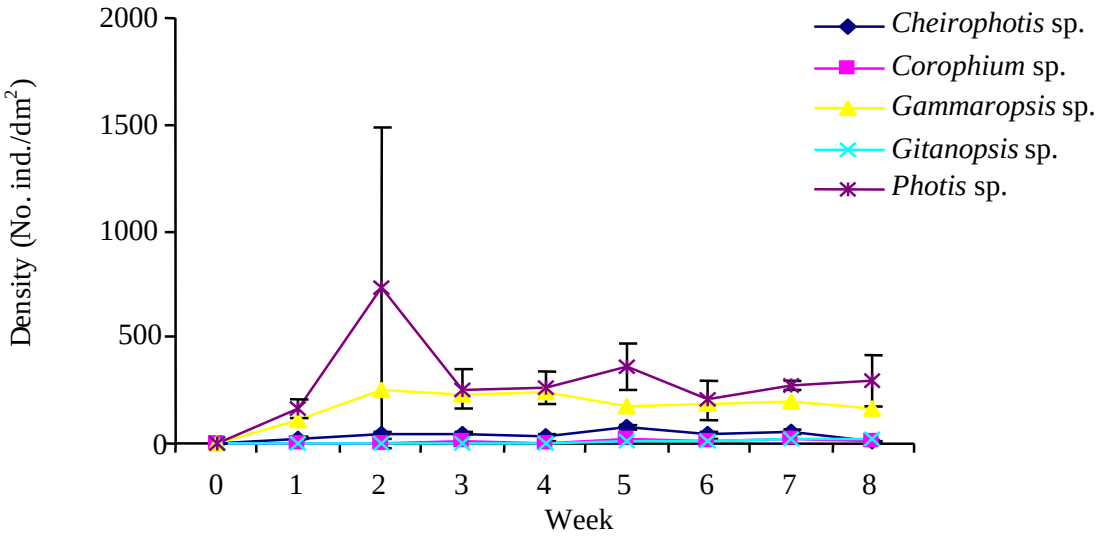
Figure 3.7. Temporal changes in the abundance (mean ± SD) of amphipods on net panels placed inside the net-cages given trash-fish feed in a fish farm at (a) Jaha (dry season) and (b) Sangga Besar (dry season).

Table 3.3. Mean density of amphipod species over eight weeks of colonization on net panels placed inside the net-cages given trash-fish feed in a fish farm at Jaha (dry season) and Sangga Besar (dry season). Standard deviation (SD) in parentheses.

	Jaha (dry season) (No. ind./dm^2)	Sangga Besar (dry season) (No. ind./dm^2)
Cheirophotis sp.	8.54 (91.89)	40.04 (12.65)
Corophium sp.	5.02 (3.52)	36.48 (9.10)
Gammaropsis sp.	1,490.33 (306.30)	194.70 (31.55)
Gitanopsis sp.	6.61 (3.41)	14.57 (6.31)
Photis sp.	840.58 (383.40)	317.37 (207.53)



(a) Jaha (dry season)



(b) Sangga Besar (dry season)

Figure 3.8. Temporal changes in the abundance (mean ± SD) of amphipod species on net panel placed inside the net-cages given trash-fish feed in a fish farm at (a) Jaha (dry season) and (b) Sangga Besar (dry season).

of colonization was 194.17 ind./dm^2 and 317.38 ind./dm^2 respectively (see Table 3.3). Unlike that in Jaha, there were no competition between *Photis* sp. and *Gammaropsis* sp. in Sangga Besar. The development rates of *Photis* sp were much higher than *Gammaropsis* sp.. On the 2nd week, density of *Photis* sp. was maximum with 729.33 ind./dm^2 as compared to 252.8 ind./dm^2 for *Gammaropsis* sp. (Figure 3.8b). However, densities of both *Photis* sp. and *Gammaropsis* sp. were reduced on the 3rd week and remained constant until the 8th week.

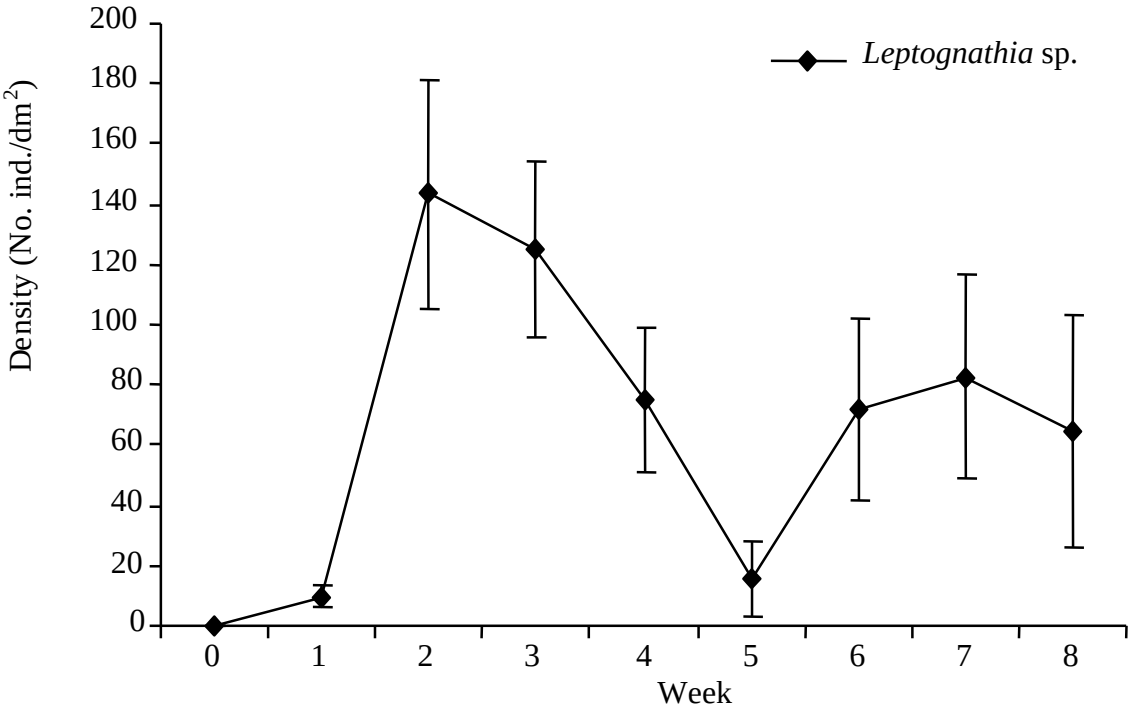
The mean density and development rates of *Cheirophotis* sp., *Corophium* sp. and *Gitanopsis* sp. were relatively lower compared to *Gammaropsis* sp. and *Photis* sp. in both estuaries.

b. Tanaidacea

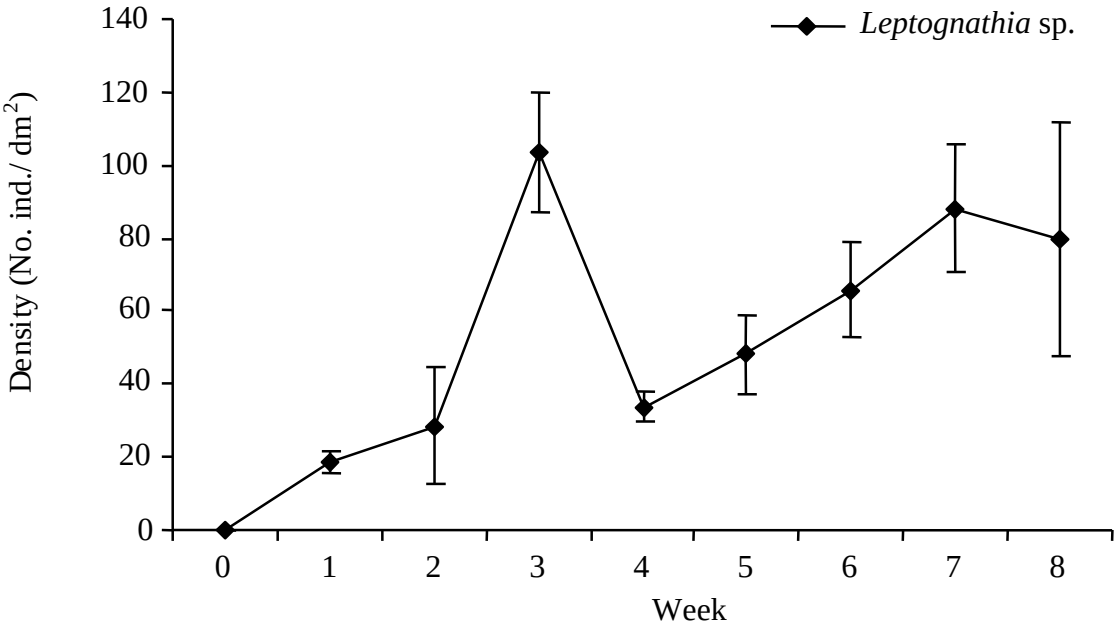
Leptognathia sp. was among the important non-sessile macrofouling organisms on net panels placed inside the net-cages given trash-fish feed. Total mean density over eight weeks of colonization was 78.67 ind./dm^2 and 58.21 ind./dm^2 respectively at Jaha and Sangga Besar. Weekly development rates of *Leptognathia* sp. were relatively slower compared to *Gammaropsis* sp. or *Photis* sp.. In Jaha, density increased rapidly to a maximum of 143.41 ind./dm^2 on 2nd week (Figure 3.9a). Similar to *Gammaropsis* sp., density of *Leptognathia* sp. decreased very rapidly to 15.42 ind./dm^2 on the 5th week, probably due to space and food competition with more dominant *Photis* sp.. In Sangga Besar, density increased to maximum of 103.38 ind./dm^2 on the 3rd week (Figure 3.9b).

c. Nematoda

The density of nematodes was among the highest on net panels placed inside the net cages given trash-fish feed. Mean density over eight weeks of colonization was 44.55 ind./dm^2 and 48.89 ind./dm^2 respectively for Jaha and Sangga Besar. In both estuaries, weekly development rates were relatively higher for the first two week,

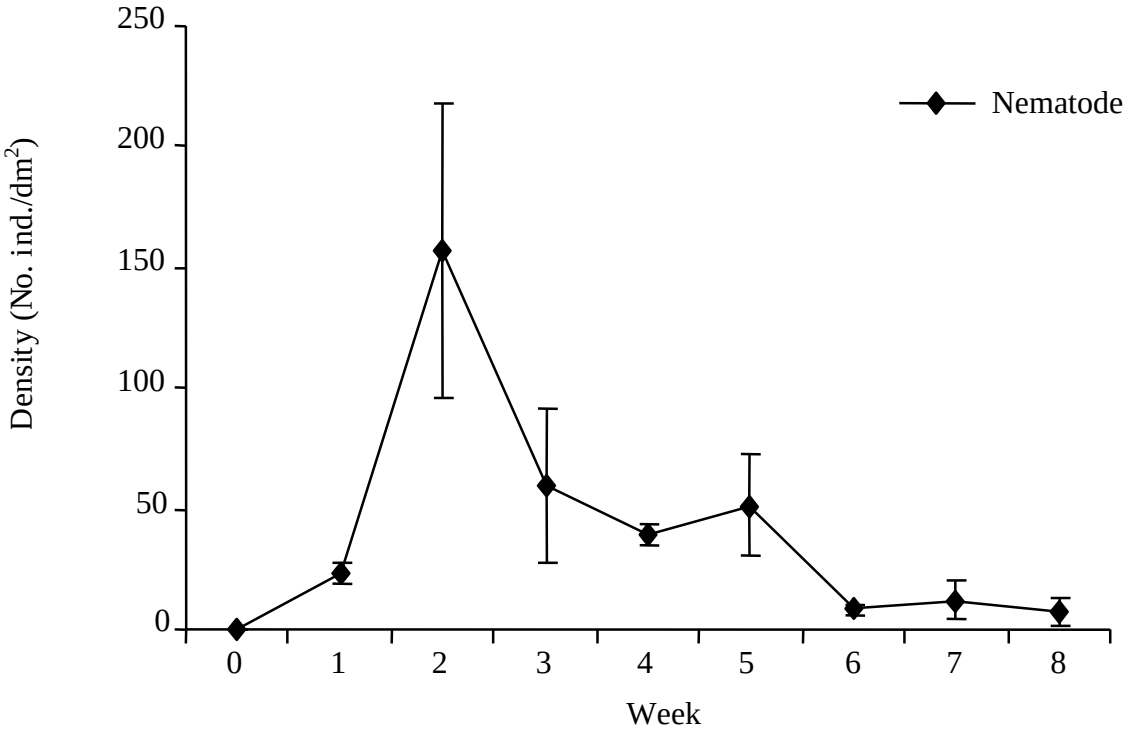


(a) Jaha (dry season)

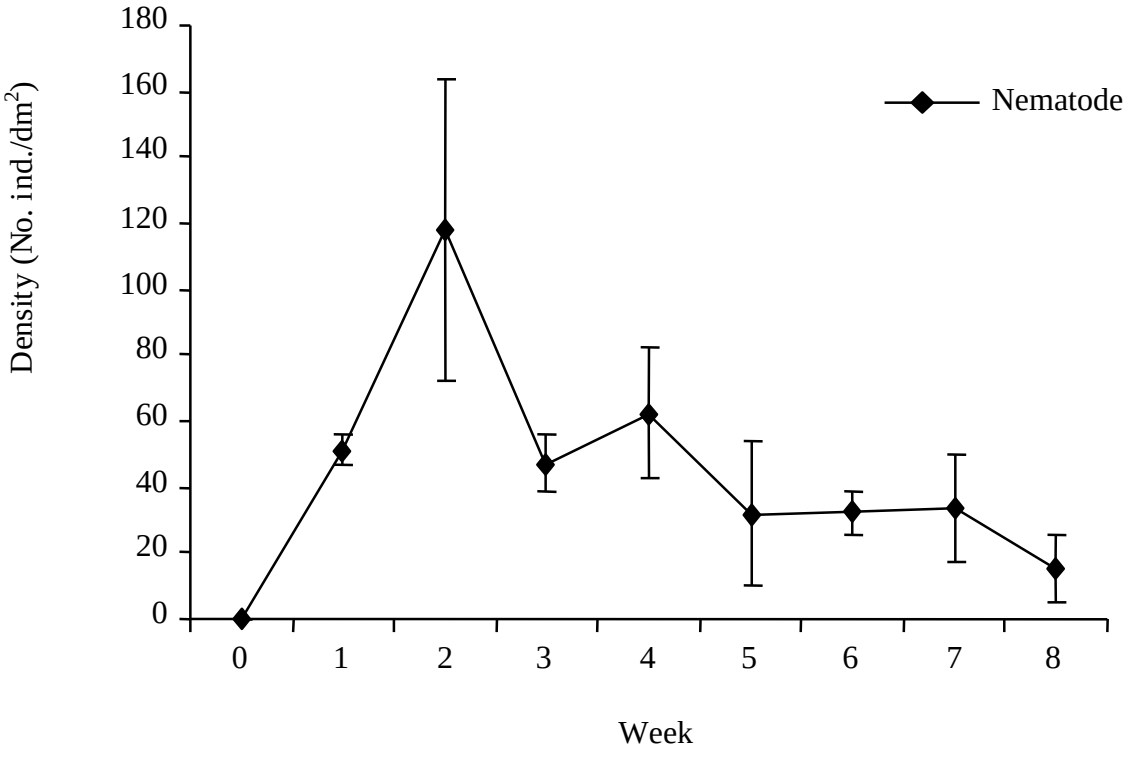


(b) Sangga Besar (dry season)

Figure 3.9. Temporal changes in abundance (mean ± SD) of *Leptognathia* sp. on net panels placed inside the net-cages given trash-fish feed in a fish farm at (a) Jaha (dry season) and (b) Sangga Besar (dry season).



(a) Jaha (dry season)



(b) Sangga Besar (dry season)

Figure 3.10. Temporal changes in the abundance (mean ± SD) of nematodes on net panels placed inside the net-cages given trash-fish feed in a fish farm at (a) Jaha (dry season) and (b) Sangga Besar (dry season).

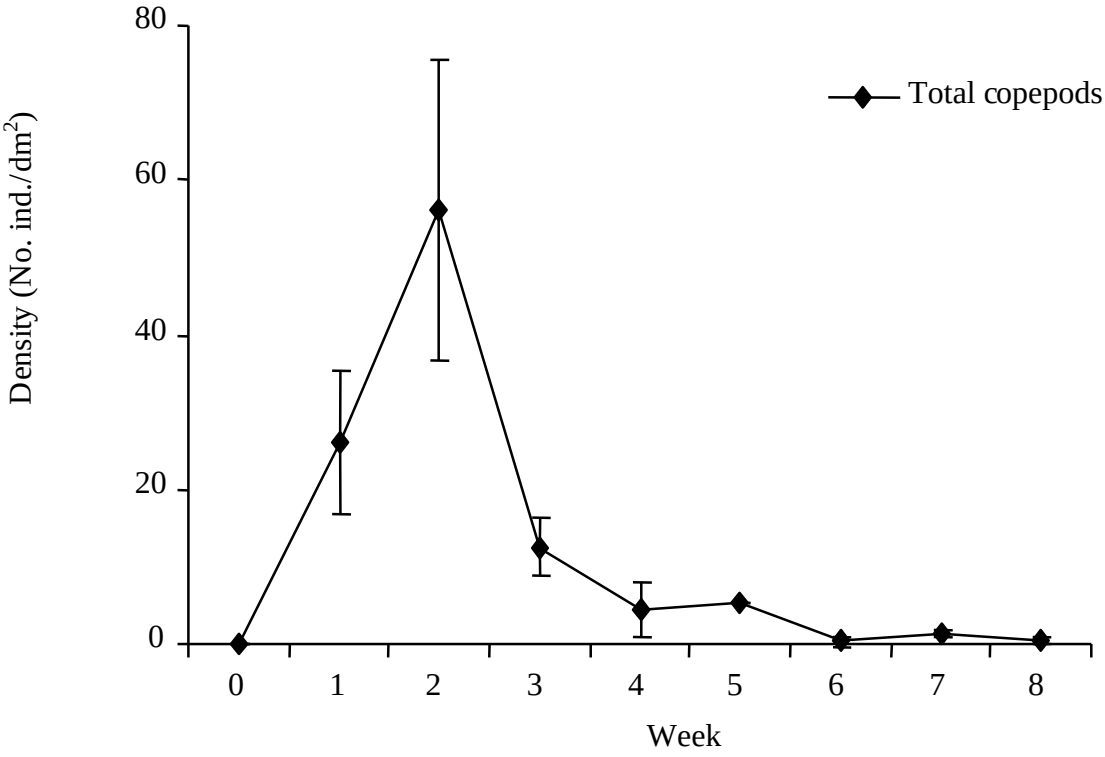
however it gradually declining thereafter. Maximum density was archived on the 2nd week with 153.21 ind./dm^2 and 118.00 ind./dm^2 respectively for Jaha (Figure 3.10a) and Sangga Besar (Figure 3.10b).

d. Copepoda

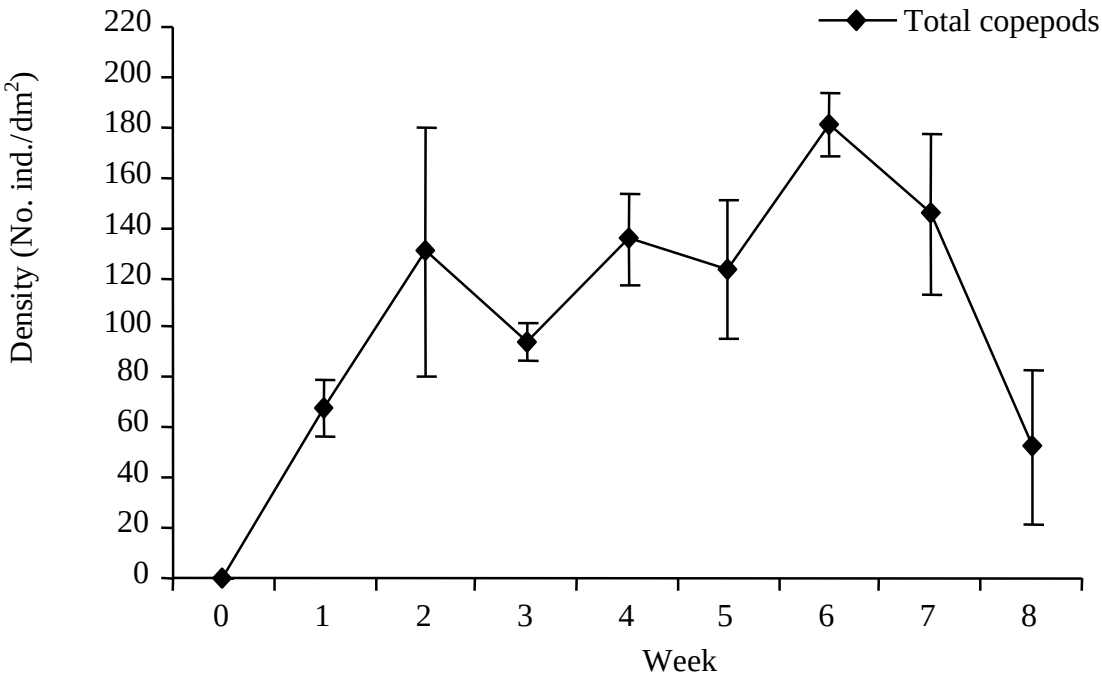
Mean density of copepods on net panels placed inside the net-cages given trash-fish feed was relatively lower than amphipods, tanaids or nematodes. Mean density over the eight weeks of colonization was relatively higher in Sangga Besar (116.16 ind./dm^2) than in Jaha (14.03 ind./dm^2). In Jaha, development rates were higher for the first two week of colonization, reaching maximum density of 56.13 ind./dm^2 on the 2nd week (Figure 3.11a). Density rapidly declined thereafter and there were almost no copepods on the following 6th – 8th weeks.

The copepods population increased much faster and constant in Sangga Besar than in Jaha. A maximum population density of 180.71 ind./dm^2 was obtained at the 6th week (Figure 3.11b). However, density gradually decreased at near end experiment similar to that in Jaha.

The copepods on net panels placed inside the net-cages given trash-fish feed were dominated by *E. acutifrons*. Mean density over eight weeks of colonization was relatively lower in Jaha (13.088 ind./dm^2) (Table 3.4) than in Sangga Besar (94.57 ind./dm^2). In Jaha, density of *E. acutifrons* increased rapidly to a maximum of 52.55 ind./dm^2 by the 2nd week but drastically dropped to only 9.71 ind./dm^2 on 3rd week (Figure 3.12a). Development rates of *E. acutifrons* were much higher and consistent in Sangga Besar. A maximum density of 152.67 ind./dm^2 obtained at the 6th week (Figure 3.12b). Population densities of other copepod species were lower (i.e. less than 5 ind./dm^2) and present occasionally on net panels.



(a) Jaha (dry season)

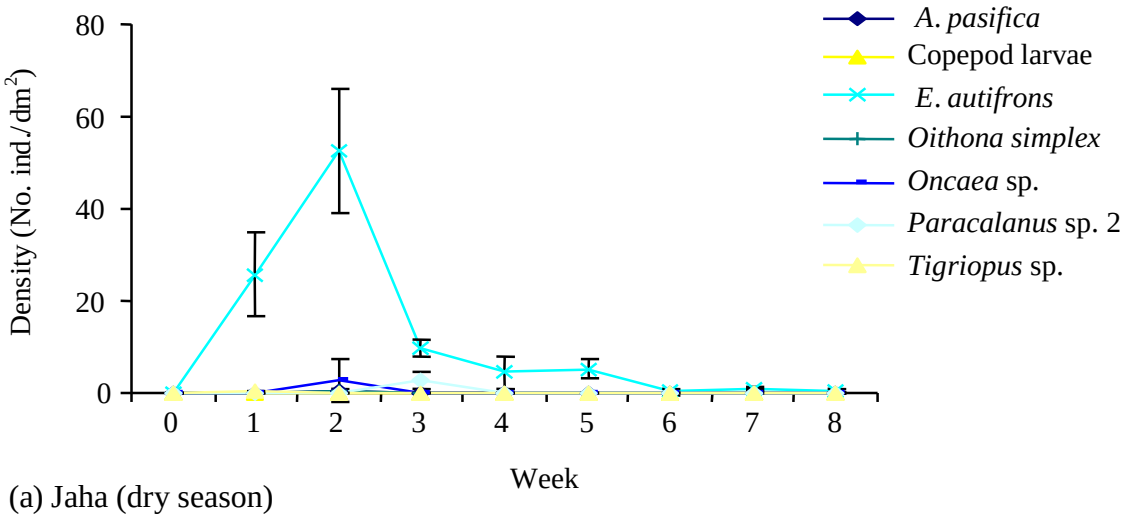


(b) Sangga Besar (dry season)

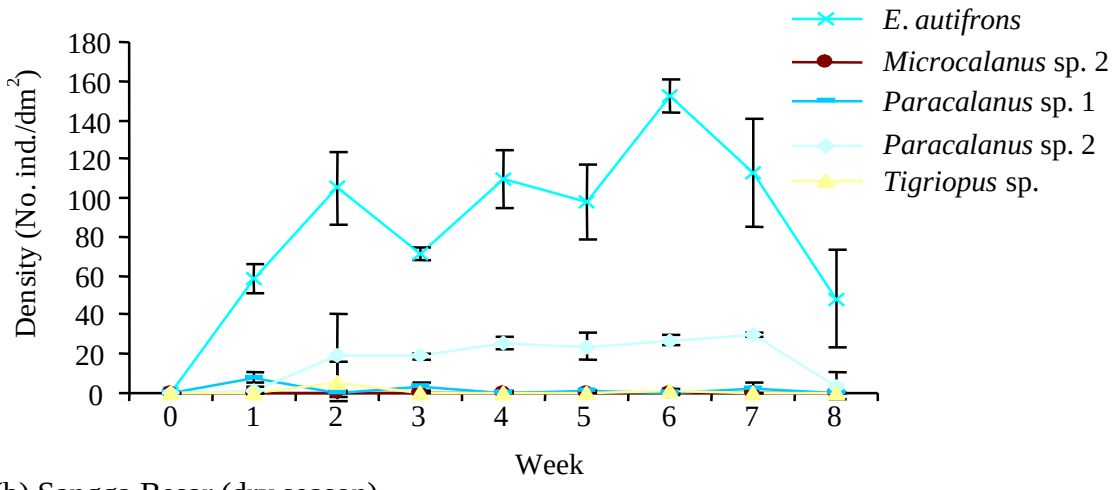
Figure 3.11. Temporal changes in abundance (mean ± SD) of copepods on net panels placed inside the net-cages given trash-fish feed in a fish farm at (a) Jaha (dry season) and (b) Sangga Besar (dry season).

Table 3.4. Mean density of copepod species over eight weeks of colonization on net panels placed inside the net-cages given trash-fish feed in a fish farm at Jaha (dry season) and Sangga Besar (dry season). Standard deviation (SD) in parentheses.

	Jaha (dry season) (No. ind./dm^2)	Sangga Besar (dry season) (No. ind./dm^2)
A. pasifica	0.06 (0.07)	0.00 (0.00)
Copepod larvae	0.02 (0.04)	0.00 (0.00)
E.autifrons	13.08 (4.19)	94.56 (15.51)
Oithona sp.	0.04 (0.08)	0.00 (0.00)
Oncaea sp.	0.34 (0.66)	0.00 (0.00)
Paracalanus sp. 2	0.36 (0.27)	18.72 (5.46)
Tigriopus sp.	0.06 (0.07)	0.97 (1.63)
Microcalanus sp. 2	0.00 (0.00)	0.02 (0.04)
Paracalanus sp. 1	0.00 (0.00)	1.89 (1.04)



(a) Jaha (dry season)



(b) Sangga Besar (dry season)

Figure 3.12. Temporal changes in the abundance (mean ± SD)of copepod species on net panels placed inside the net-cages given trash-fish feed in a fish farm at (a) Jaha (dry season) and (b) Sangga Besar (dry season).

e. Polychaeta and others

The density of polychaete and other taxa such as balanoid larvae, brachyuran megalopae and isopods were lower. Density was relatively less than 10 ind./dm^2 per net panels and present occasionally.

3.2.5. Macrofouling Community of Net Panels Suspended Inside Net-Cages (Trash-Fish Feed, Wet Season)

3.2.5.1. Species Composition of Sessile and Non-Sessile Associates

The species compositions of sessile macrofouling and non-sessile organisms on net panels placed inside the net-cages given trash-fish feed at Jaha during the wet season were not significantly different from that of the dry season. The 12-weeks biofouling study during the wet season shows that *Polysiphonia* sp., *Enteromorpha clathrata*, *Lyngbya* sp., *Xenostrobus mangle*, anthozoans, *Plumularia* sp. and *Balanus amphitrite* were the same species encountered in the dry season.

Species compositions of non-sessile organisms on net panels placed inside the net-cages given trash-fish feed were not seasonally different but their density changed significantly. There were 23 associated non-sessile macrofouling species enumerated during the wet season. *Paracalanus* sp. 1, Saphierella-like copepodid and Terebellidae were occasionally present during the wet season but not in the previous dry season. However, *Oithona simplex* and brachyuran megalopae were not encountered during the wet season.

3.2.5.2. Depth Distribution and Percentage Cover of Sessile Macrofouling Organisms

The eight-week comparison between wet and dry seasons in Jaha indicates that depth preferences of the sessile macrofouling organisms on net panels placed inside the net-cages given trash-fish feed were relatively similar, although their percentage cover was different. In the wet season (Figure 3.13a), mean percentage cover (27.1%) of

Polysiphonia sp. at the upper stratum was significantly ($P < 0.001$) lower than in the dry season (see Figure 3.5a) where there were no colonies developed at the middle and bottom strata. Mean percentage cover of *Plumularia* sp. at the upper (58.7%), middle (91.9%) and bottom (98.5%) strata was significantly ($P < 0.05$) higher than in the dry season (see Appendix 4).

Mean percentage covers of anthozoans and *B. amphitrite* were significantly ($P < 0.05$) lower during the wet season; maximum cover of anthozoans at the middle stratum was 5.8% while maximum for *B. amphitrite* was 4% at the upper stratum respectively (see Figure 3.13a). Interestingly, mean percentage cover of *X. mangle* at the upper stratum (5.6%) was significantly ($P < 0.05$) higher during the wet season than in the dry season (see Figure 3.5a).

3.2.5.3. Temporal Change in Species Composition and Percentage Cover of Sessile Macrofouling Organisms

Plumularia sp. was the first observed sessile macrofouler on net panels placed inside the net-cages given trash-fish feed during the wet season (Figure 3.13b) as similarly observed in the dry season (see Figure 3.6a). However, development rates were much higher, achieving 100% cover at all depth strata after one week of immersion. At the upper stratum, *Plumularia* sp. cover decreased to 13.5% on the 6th week, when *Polysiphonia* sp. and *X. mangle* increased to 31.4% and 10.6% respectively. Percentage cover of *Plumularia* sp. was more stable at the middle or bottom stratum as there was no significant reduction until the 12th week.

Development rates of other macrofouling organisms were relatively slower during the wet season. *Polysiphonia* sp. started to develop on 5th week and maximum cover of 73.2% at the upper stratum was obtained on the 8th week. *X. mangle* started to develop on the 3rd or 4th week and maximum cover of 46.2% at the upper stratum was obtained

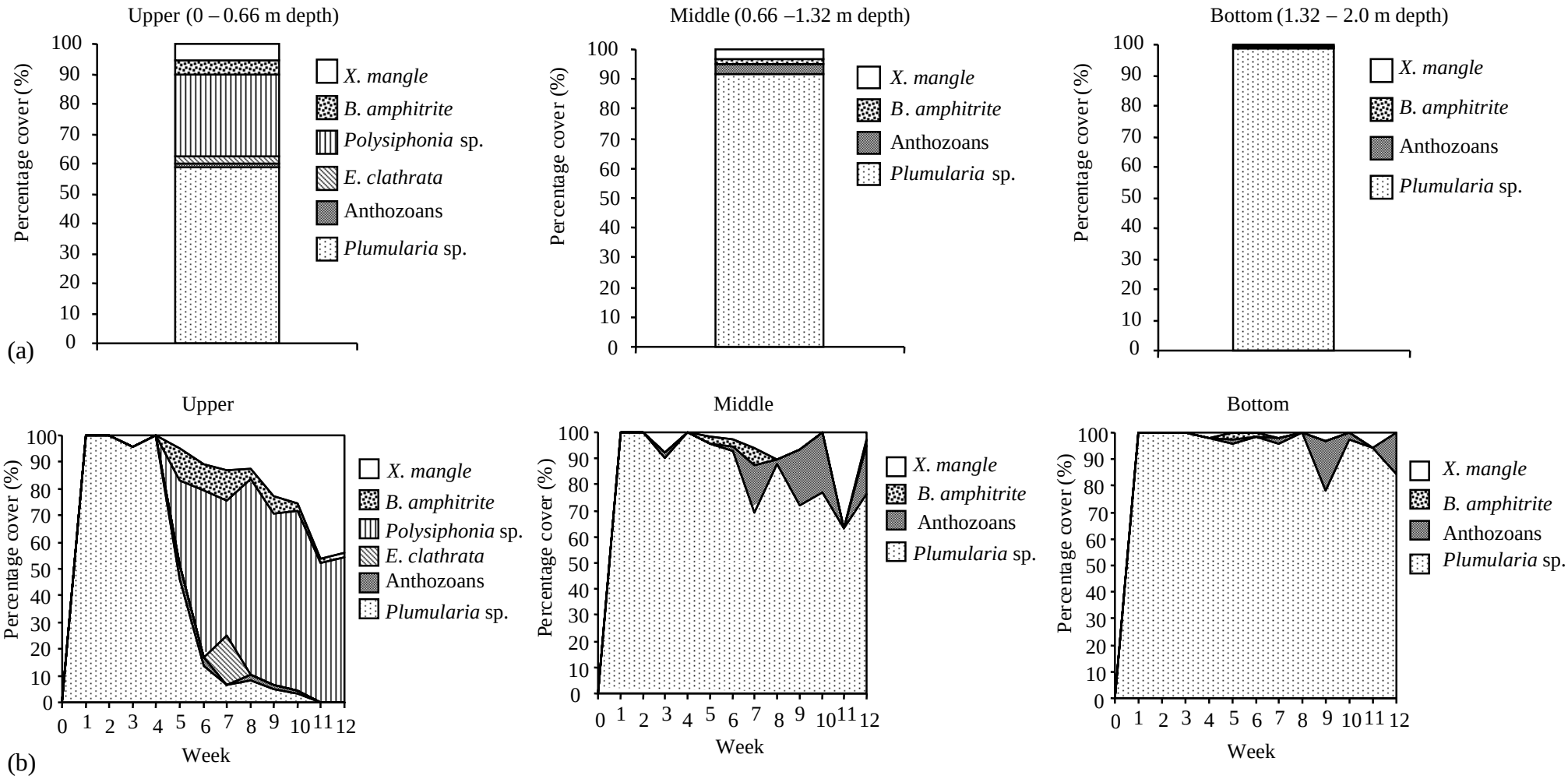


Figure 3.13. Depth distribution and percentage cover of sessile macrofouling organims (upper, middle & bottom strata) over eight weeks of colonization on net panels placed inside the net-cages given trash-fish feed (a), and temporal changes in species composition and percentage cover during the wet season in a fish farm at Jaha (b).

at 11th week (see Figure 3.13b). The weekly development rates of anthozoans and *B. amphitrite* were also slower in the wet season; a maximum cover of 22.8% at the middle stratum on the 10th week for anthozoans and maximum cover of 11.8% at the upper stratum on the 5th week for *B. amphitrite*. *E. clathrata* was rarely present at the upper strata and there was totally no *Lyngbya* sp. during the wet season.

3.2.5.4. Temporal Change in Species Composition and Abundance of Non-Sessile Associates

a. Amphipoda

The eight-week comparison between wet and dry seasons at Jaha indicate that mean total density of amphipods on net panels placed inside the net-cages given trash-fish feed were significantly ($P < 0.01$) lower during the wet season (701.23 ind./dm^2) than in the dry season (2,348.38 ind./dm^2). Weekly development rates were slower, maximum density of 1,997.53 ind./dm^2 was obtained at the 12th week (Figure 3.14) as compared to the maximum density obtained at the 4th week during the dry season (see Figure 3.7a).

Mean density of *Gammaropsis* sp. (512.78 ind./dm^2) and *Photis* sp.(178.03 ind./dm^2) on net panels placed inside the net-cages given trash-fish feed over eight weeks of colonization during the wet season (Table 3.5) were significantly ($P < 0.05$) lower than during the dry season (see Table 3.3). As in the dry season, *Gammaropsis* sp. was an important early colonizer of net panels during the wet season (Figure 3.15), however weekly development rates were much slower than in the dry season (see Figure 3.8a). A maximum density of 718.77 ind./dm^2 on the 6th week was relatively lower than that of the nearly maximum density obtained on the 2nd week during the dry season.

Development rates of *Photis* sp. were relatively slower during the wet season. Its density surpassed that of the *Gammaropsis* sp. density on the 8th week and a maximum density of 1,640.16 ind./dm^2 obtained at the 12th week. Mean density and

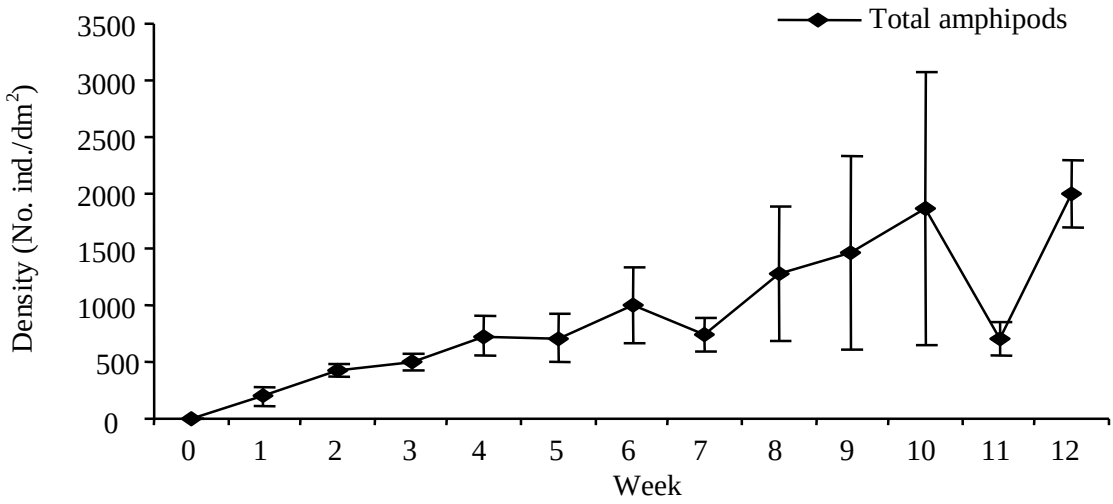


Figure 3.14. Temporal changes in the abundance (mean ± SD) of amphipods on net panels placed inside the net-cages given trash-fish feed during the wet season in a fish farm at Jaha.

Table 3.5. Mean density of amphipods species over eight weeks of colonization on net panels placed inside the net-cages given trash-fish feed during the wet season in a fish farm at Jaha. Standard deviation (SD) in parentheses.

	Jaha (wet season) (No. ind./dm^2)
Cheirophotis sp.	0.00 (0.00)
Corophium sp.	2.91 (2.74)
Gammaropsis sp.	512.78 (101.39)
Gitanopsis sp.	6.43 (4.15)
Photis sp.	178.03 (77.36)

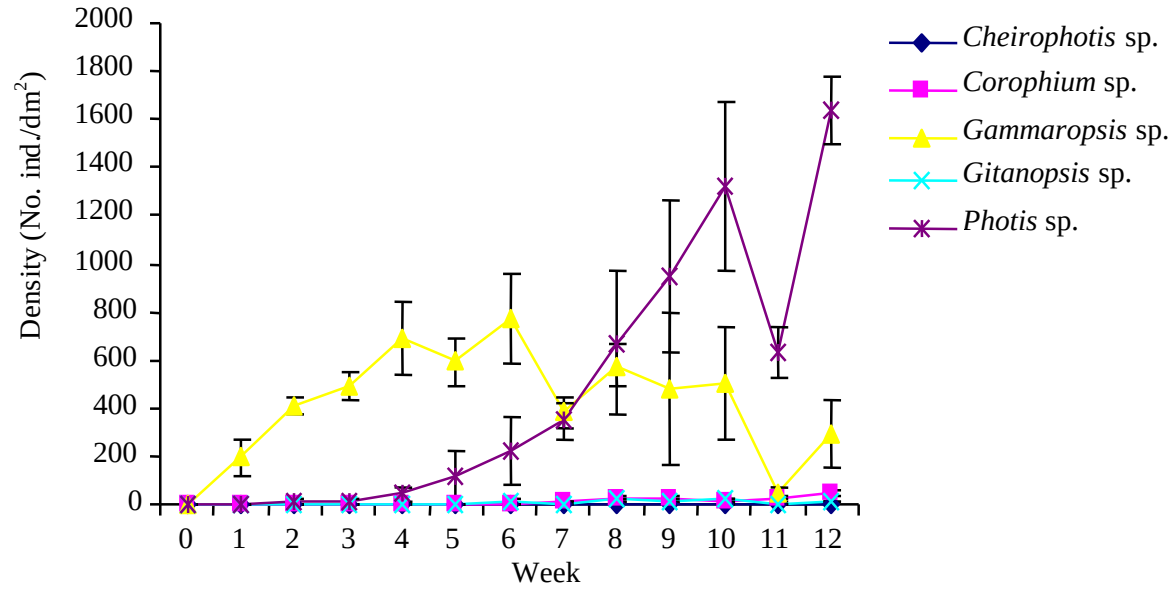


Figure 3.15. Temporal changes in the abundance (mean ± SD) of amphipods species on net panels placed inside the net-cages given trash-fish feed during the wet season in a fish farm at Jaha.

weekly development rates of other amphipods species were generally low during the wet season (i.e. less than 10 ind./dm^2) (see Appendix 5).

b. Tanaidacea

The eight-week comparison between wet and dry seasons in Jaha indicates that mean density of *Leptognathia* sp. on net panels placed inside the net-cages given trash-fish feed was significantly ($P < 0.05$) lower during the wet (30.80 ind./dm^2) than in the dry season. Weekly development rates were slower than in the dry season. A maximum density of 77.02 ind./dm^2 on the 8th week (Figure 3.16) was relatively low than the maximum density obtained on the 2nd week during the dry season (see Figure 3.9a).

c. Nematoda

The eight-week comparison between wet and dry seasons at Jaha indicates that mean density of nematode on net panels placed inside the net-cages given trash-fish feed was significantly higher during the wet season (93.02 ind./dm^2) than in the dry season at Jaha. The weekly development rates were high than during the wet season. A maximum density of 189.57 ind./dm^2 on the 5th week (Figure 3.17), was significantly higher ($P < 0.05$) than the maximum archived at the 2nd week during the dry season (see Figure 3.10a).

d. Copepoda

The eight-week comparison between wet and dry seasons at Jaha indicates that mean total density of copepods population on net panels placed inside the net cages given trash-fish feed was significantly higher during the wet season (24.98 ind./dm^2) than in the dry season. The weekly development rates were relatively higher and consistent during the wet season (Figure 3.18) than in the dry season (see Figure 3.11a). A maximum density of 55.70 ind./dm^2 obtained at the 9th week.

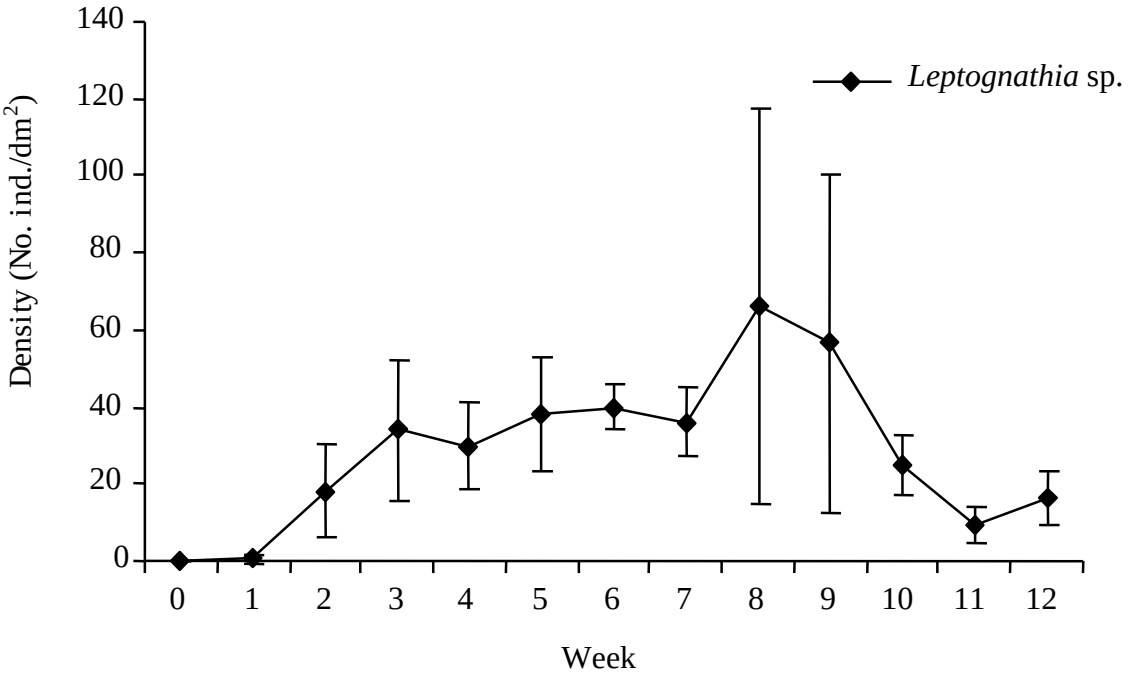


Figure 3.16. Temporal changes in abundance (mean ± SD) of *Leptognathia* sp. on net panels placed inside the net-cages given trash-fish feed during the wet season in a fish farm at Jaha.

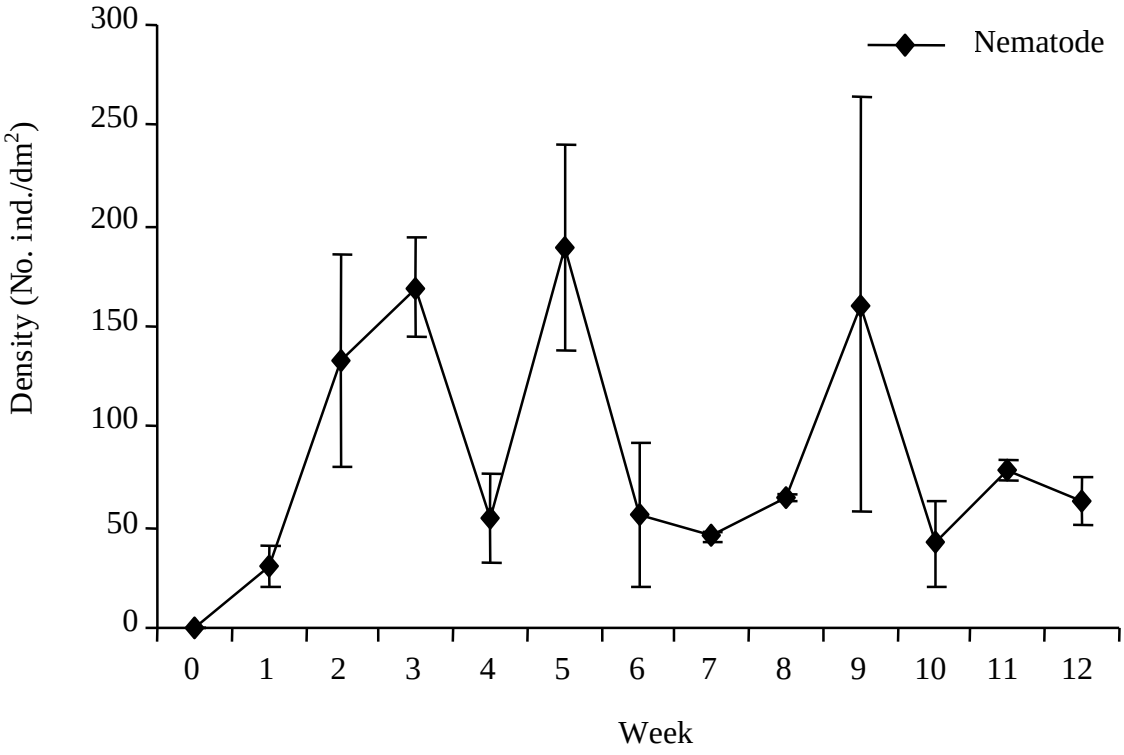


Figure 3.17. Temporal changes in abundance (mean ± SD) of nematode on net panels placed inside the net-cages given trash-fish feed during the wet season in a fish farm at Jaha.

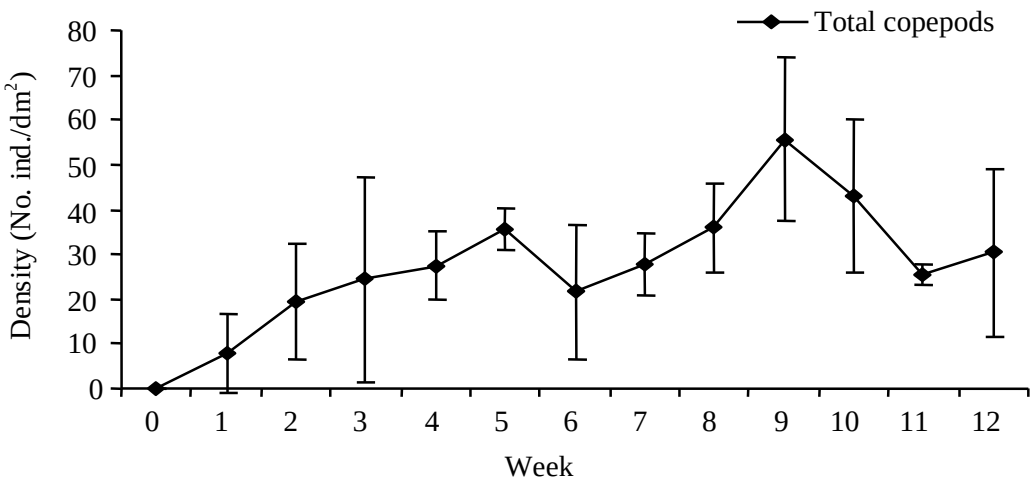


Figure 3.18. Temporal changes in abundance (mean ± SD) of copepods on net panels placed inside the net-cages given trash-fish feed during the wet season in a fish farm at Jaha.

Table 3.6. Mean density of copepod species over eight weeks of colonization on net panels placed inside the net-cages given trash-fish feed during the wet season in a fish farm at Jaha River estuary. Standard deviation (SD) in parentheses.

	Jaha (wet season) (No. ind./dm^2)
A. pasifica	0.03 (0.05)
Copepod larvae	0.12 (0.21)
E. acutifrons	24.25 (10.05)
Oncaea sp.	0.09 (0.09)
Paracalanus sp 1.	0.18 (0.24)
Paracalanus sp 2.	0.06 (0.11)
Saphierella-like copepodid	0.13 (0.05)
Tigriopus sp.	0.06 (0.93)

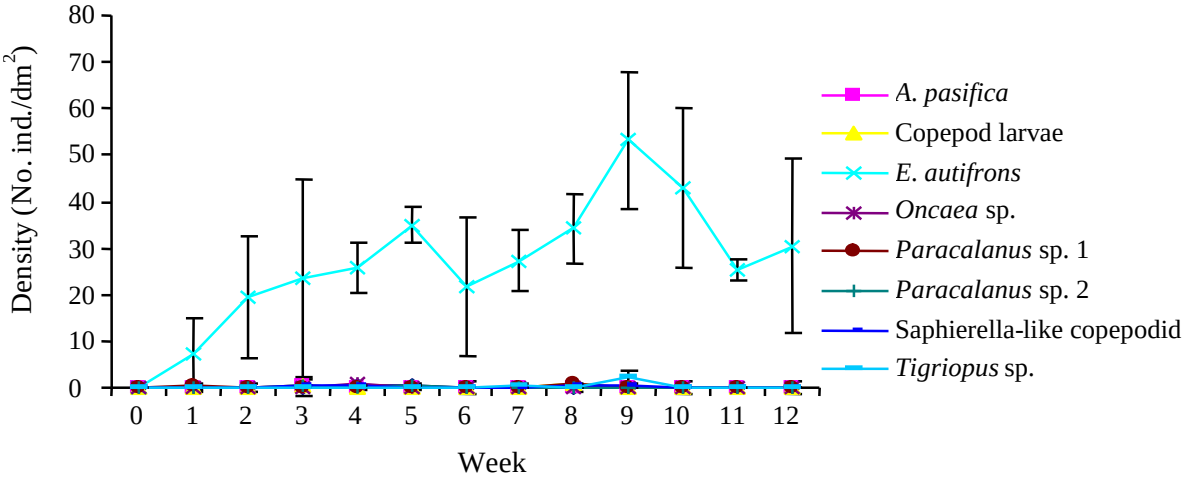


Figure 3.19. Temporal changes in abundance (mean ± SD) of copepods species on net panels placed inside the net-cages given trash-fish feed during the wet season in a fish farm at Jaha.

Similar to the result of the dry season, the wet season copepods on net panels placed inside the net-cages given trash-fish feed were dominated by *E. acutifrons*. However, mean density of 24.25 ind./dm^2 (Table 3.6) was significantly ($P < 0.05$) higher than in the dry season (see Table 3.4). The weekly development rates of *E. acutifrons* were higher during the wet season (Figure 3.19) than in the dry season (see Figure 3.12a). A maximum density of 53.23 ind./dm^2 obtained at the 9th week. Densities and development rates of other copepods species were relatively lower and present occasionally similar to that observation during the dry season.

e. Polychaeta and others

The density of polychaetes and other taxa on net panels placed inside the net-cages given trash-fish feed was not seasonally different as their numbers were relatively lower and occasionally encountered as was observed in the dry season.

3.2.6. Macrofouling Community of Net Panels Suspended Inside Net-Cages (Pellet Feed, Dry and Wet Season)

3.2.6.1. Species Composition of Sessile and Non-Sessile Associates

In both seasons at Jaha, species composition of sessile macrofouling organisms on net panels placed inside the net-cages given pellet feed were similar to that given trash-fish feed. Organic materials were accumulated on net panels similar to that in net-cages given trash-fish feed. In Sangga Besar, *Lyngbya* sp. was present inside the net-cages given pellet feed, however it was not encountered in net-cages given trash-fish feed. Other species were similar between pellet and trash-fish feed.

During the dry season at Jaha, non-sessile organisms on net panel placed inside the net-cages given pellet feed comprised 24 species including unidentified copepod larvae and polychaete juveniles (see Table 3.2). *Microcalanus* sp. 1, *Paracalanus* sp. 1 and Terebellidae sp. were encountered but not in net-cages given

trash-fish feed. However, *Paracalanus* sp. 2 was not found in pellet feed given net-cages. In the wet season, number of non-sessile species was 26, these including the unidentified copepod larvae and polychaeta juvenile. *Microcalanus* sp. 1, *Microcalanus* sp. 2 and brachyuran megalopae were encountered however this species not in trash-fish feed given net-cages.

A total of 18 non-sessile species were encountered on net panels placed inside net-cages given pellet feed in Sangga Besar, however the dominant species were similar between pellet and trash-fish feed. There were no Terebellidae, balanoid larvae and *Microcalanus* sp. 2. in pellet cages while brachyuran megalopae and copepod larvae were in pellet feed but not in net-cages given trash-fish feed.

3.2.6.2. Depth Distribution and Percentage Cover of Sessile Macrofouling Organisms

During the dry season at Jaha, depth distribution and percentage cover of *Polysiphonia* sp., *Plumularia* sp., anthozoans, *B. amphitrite*, and *X. mangle* was relatively similar between pellet (Figure 3.20a) and trash-fish feed (see Figure 3.5a). However, mean percentage cover of *Lyngbya* sp. was significantly ($P < 0.05$) higher at all depth strata of net panels placed inside the net-cages given pellet feed than in trash-fish cages. It was 7.6% at upper stratum and 6.2% at middle and bottom stratum respectively.

During the wet season at Jaha, depth distribution and percentage cover of *Polysiphoni*a sp., *Plumularia* sp., anthozoans, *B. amphitrite*, and *Lyngbya* sp. were relatively similar between pellet (Figure 3.20b) and trash-fish feed (see Figure 3.13a). However, *X. mangle* was significantly ($P < 0.05$) higher inside the pellet given net-cages particularly at the upper stratum (32.1%) as compared to only 17.7% in trash- fish feed given net-cages (see Appendix 4).

In Sangga Besar, depth distribution of sessile macrofouling species was relatively similar between pellet (Figure 3.20c) and trash-fish (see Figure 3.5b) cages, although

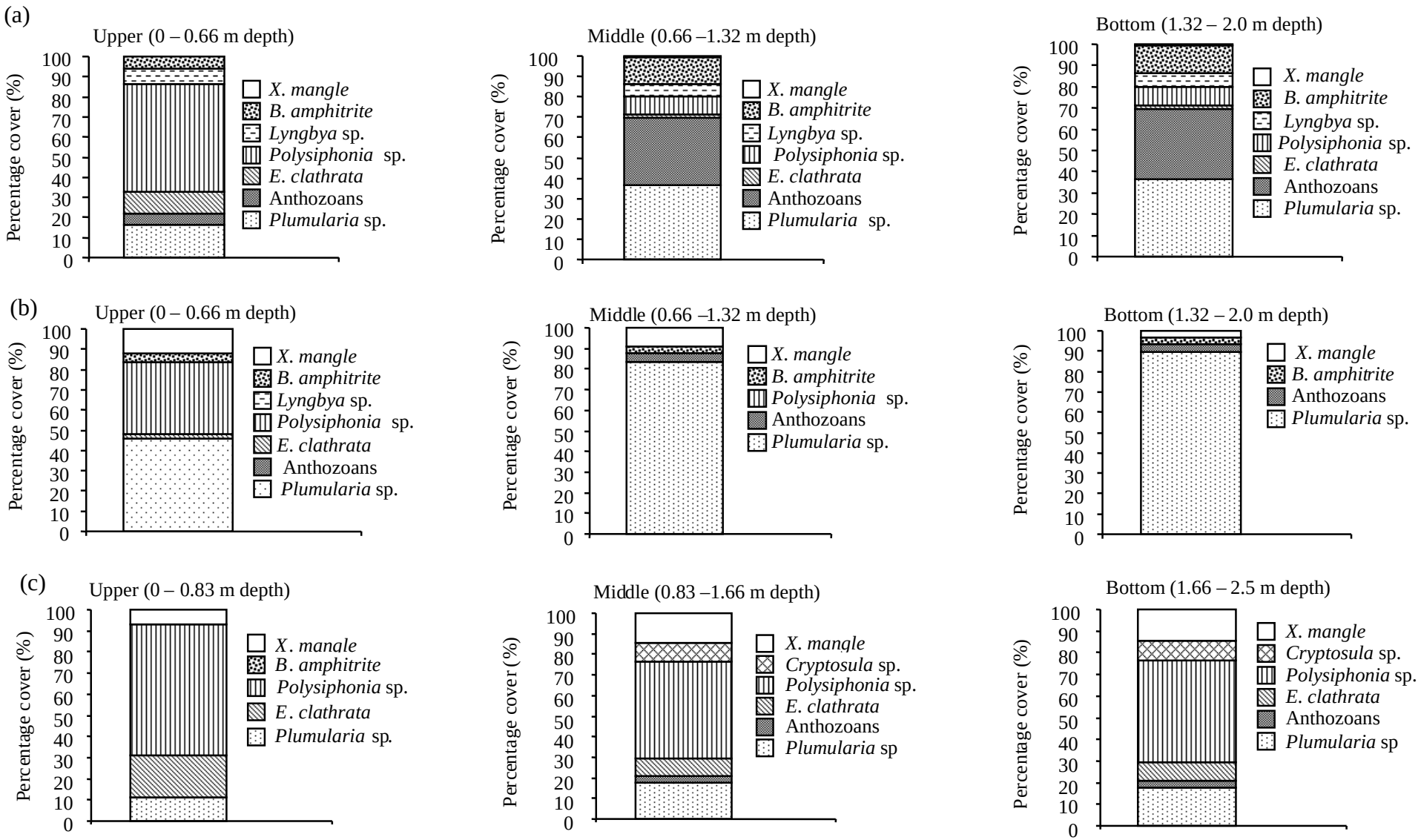


Figure 3.20. Depth distribution and percentage cover of sessile macrofouling organisms (upper, middle & bottom strata) over eight weeks of colonization on net panels placed inside the net-cages given pellet feed in a fish farm at (a) Jaha (dry season), (b) Jaha (wet season) and (c) Sangga Besar (dry season).

their percentage cover was different. Mean percentage cover of *Polysiphonia* sp. and *X. mangle* at all depth strata was significantly ($P < 0.05$) higher than in net-cages given trash-fish feed. Mean percentage covers of *Plumularia* sp. and *Cryptosula* sp. was significantly ($P < 0.05$) lower in pellet than in trash-fish feed given net-cages.

3.2.6.3. Temporal Change in Species Composition and Percentage Cover of Sessile Macrofouling Organisms

In the dry season at Jaha, development trend of *Plumularia* sp., *Polysiphonia* sp., *E. clathrata*, anthozoans and *B. amphitrite* in net-cages given pellet feed (Figure 3.21a) were similar to that in trash-fish given net-cages (see Figure 3.6a). However, *Lyngbya* sp. appeared on the 2nd week with percentage cover of 41.3%, 29.3% and 32.7% at the upper, middle and bottom strata respectively.

In the wet season at Jaha, net panels placed inside the net-cages given pellet feed were almost entirely dominated by *Plumularia* sp. (Figure 3.21b), a situation quite similar to that in net-cages given trash-fish feed (see Figure 3.13b). However, colonization rates of *Polysiphonia* sp. and *X. mangle* were more aggressive than in trash-fish feed. A maximum cover of 65.4% was achieved by *Polysiphonia* sp. on 7th week and *X. mangle* achieved 72.3% cover on 11th week at the upper stratum respectively. These cover were significantly ($P < 0.05$) higher than in net-cages given trash-fish feed. Colonization trend of other species were similar between pellet and trash-fish feed.

In Sangga Besar, the early colonizers of the net panels placed inside the net-cages given pellet feed were *Plumularia* sp. and *E. clathrata* (Figure 3.21c), these was similar to that in net-cages given trash-fish feed (see Figure 3.6b). Other species were started to develop after the 2nd week, however their aggressive development resulted in the gradual reduction of *Plumularia* sp. and *E. clathrata*. Unlike that in

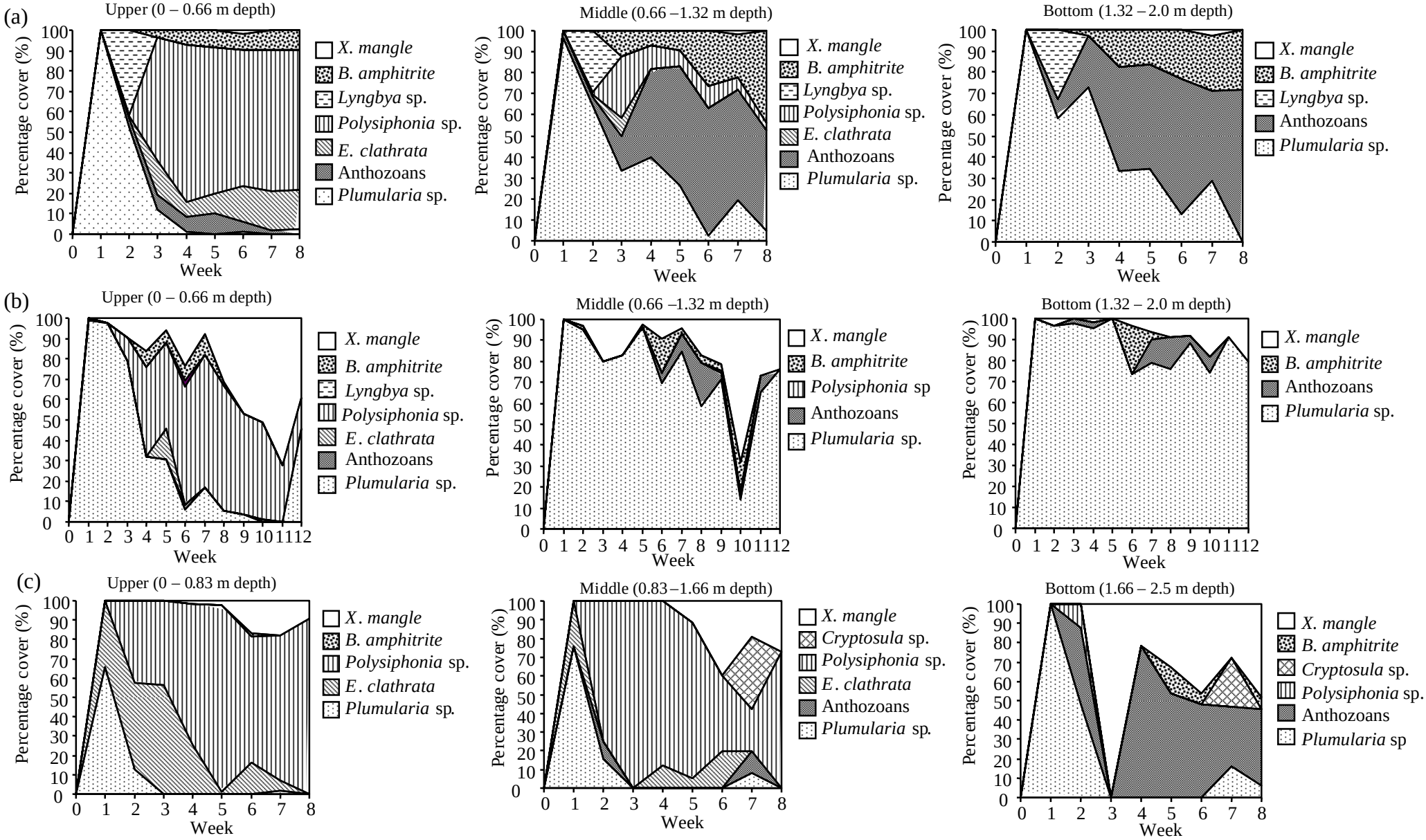


Figure 3.21. Temporal changes in species composition and percentage cover of sessile macrofouling organisms at the upper, middle and bottom strata of net panels placed inside the net-cages given pellet feed in a fish farm at (a) Jaha (dry season), (b) Jaha (wet season) and (c) Sangga Besar (dry season only).

trash-fish feed, *Cryptosula* sp. were occasionally present with lower weekly percentage cover on net panels placed inside net-cages given pellet feed.

3.2.6.4. Abundance and Temporal Change in Species Composition of Non-Sessile Associates

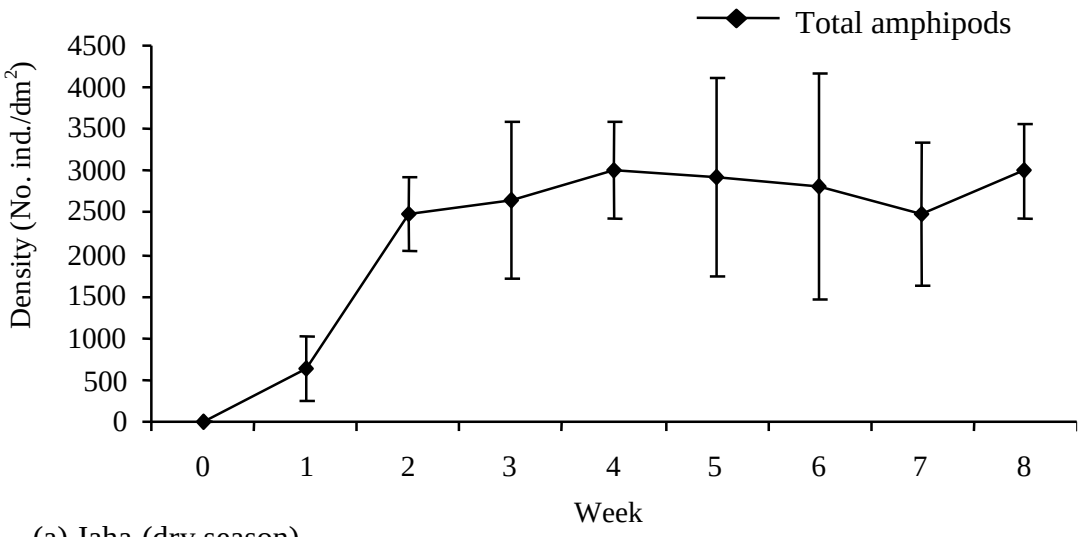
a. Amphipoda

A total mean density of amphipods on net panels placed inside the net-cages given pellet feed during the dry (2,500.32 ind./dm^2) and wet (705.23 ind./dm^2) season in Jaha was not significantly ($P > 0.05$) different to that in trash-fish feed given net-cages for both seasons. In Sangga Besar, mean total density of 1,427.23 ind./dm^2 was significantly ($P < 0.05$) higher than that in net-cages given trash-fish feed .

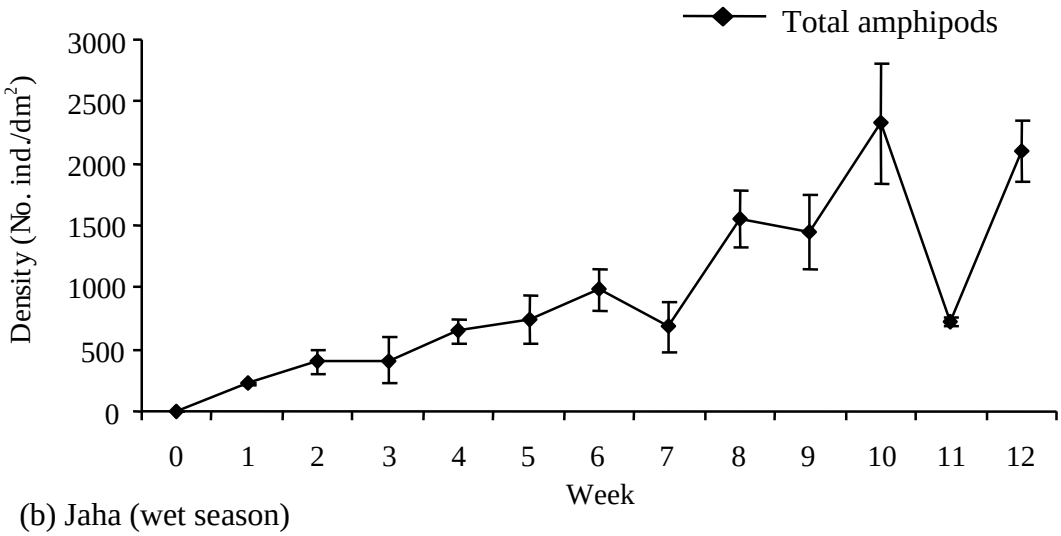
The weekly development rates of amphipods on net panels placed inside the net-cages given pellet feed during the dry (Figure 3.22a) and wet (Figure 3.22b) season at Jaha was very much similar to that in net-cages given trash-fish feed for both dry (see Figure 3.7a) and wet (see Figure 3.14) seasons. In the dry season, a maximum density of 3,019.57 ind./dm^2 was obtained on the 4th week, while in the wet season maximum of 2,323.97 ind./dm^2 obtained at the 10th week.

In Sangga Besar, weekly development rates of amphipods on net panels placed inside the net-cages given pellet (Figure 3.22c) were higher than in trash-fish cages (see Figure 3.7b). The maximum density of 2,306.61 ind./dm^2 at the 6th week was almost four times higher than in net-cages given trash-fish feed.

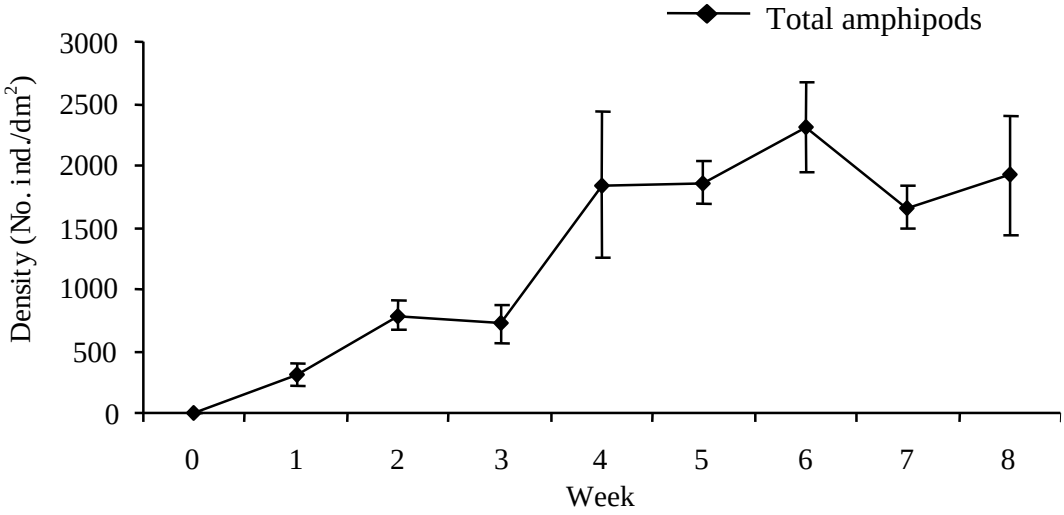
At Jaha, the densities by *Gammaropsis* sp. and *Photis* sp. on net panels placed inside the net-cages given pellet feed (Table 3.7) and trash-fish feed were not significantly ($P > 0.05$) different between the dry (see Table 3.3) and wet (see Table 3.5) seasons. During the dry season, densities of *Gammaropsis* sp. and *Photis* sp. on net panels placed inside the net-cages given pellet feed were 1,377.25 ind./dm^2 and 1,044.79



(a) Jaha (dry season)



(b) Jaha (wet season)



(c) Sangga Besar (dry season)

Figure 3.22. Temporal changes in the abundance (mean ± SD) of amphipods on a net panels placed inside the net-cages given pellet feed in a fish farm at (a) Jaha (dry season), (b) Jaha (wet season) and (c) Sangga Besar (dry season).

ind./dm^2 respectively, while in the wet season, their densities were 529.99 ind./dm^2 and 170.94 ind./dm^2 respectively (see Appendix 5).

In Sangga Besar, net panels placed inside the net-cages given pellet feed were dominated by *Photis* sp., similar to that in net-cages given trash-fish feed. Mean density at 1,114.27 ind./dm^2 (see Table 3.7) was significantly ($P < 0.05$) higher than that in net-cages given trash-fish feed (see Table 3.3). Mean density (223.16 ind./dm^2) of *Gammaropsis* sp. was not significant ($P > 0.05$) different to the density in net-cages given trash-fish feed.

In both seasons and estuary, population density of other amphipods species was not significantly different between pellet and trash-fish feed. Their population was relatively lower compared to *Gammaropsis* sp. and *Photis* sp.. *Gammaropsis* sp. and *Photis* sp. were important colonizers of the net panels placed inside the net-cages given pellet feed during the dry (Figure 3.23a) and wet (Figure 3.23b) season at Jaha. The weekly development trend and rates were relatively similar to that in trash-fish cages for both dry (see Figure 3.8a) and wet seasons (see Figure 3.15).

In Sangga Besar, development rates of *Photis* sp. in pellet cages (Figure 3.23c) were higher than in trash-fish cages (see Figure 3.8b). The maximum density of 1966.91 ind./dm^2 obtained on the 6th week.

In both seasons and estuary, development rates of other amphipods species such *Cheirophotis* sp., *Corophium* sp. and *Gitanopsis* sp. were generally slower similar to that in net-cages given trash-fish feed.

b. Tanaidacea

During the dry season at Jaha, mean density of *Leptognathia* sp. in net-cages given pellet (91.00 ind./dm^2) was significantly ($P < 0.05$) higher than that in trash-fish cages. In the wet season, mean density of 54.12 ind./dm^2 was higher than in trash-fish cages,

Table 3.7. Mean density of amphipod species over eight weeks of colonization on net panels placed inside the net-cages given pellet feed at Jaha (dry and wet season) and Sangga Besar (dry season only). Standard deviation (SD) in parentheses.

	Jaha (dry season) (No. ind./dm^2)	Jaha (wet season) (No. ind./dm^2)	Sangga Besar (dry season) (No. ind./dm^2)
Cheirophotis sp.	8.15 (4.65)	0.03 (0.00)	40.04 (9.73)
Corophium sp.	5.94 (4.33)	5.20 (2.28)	10.40 (4.76)
Gammaropsis sp.	1377.25 (228.45)	529.99 (95.36)	223.16 (22.36)
Gitanopsis sp.	4.18 (1.78)	5.18 (2.22)	9.78 (4.75)
Photis sp.	1044.79 (327.88)	170.94 (109.71)	1114.27 (164.17)

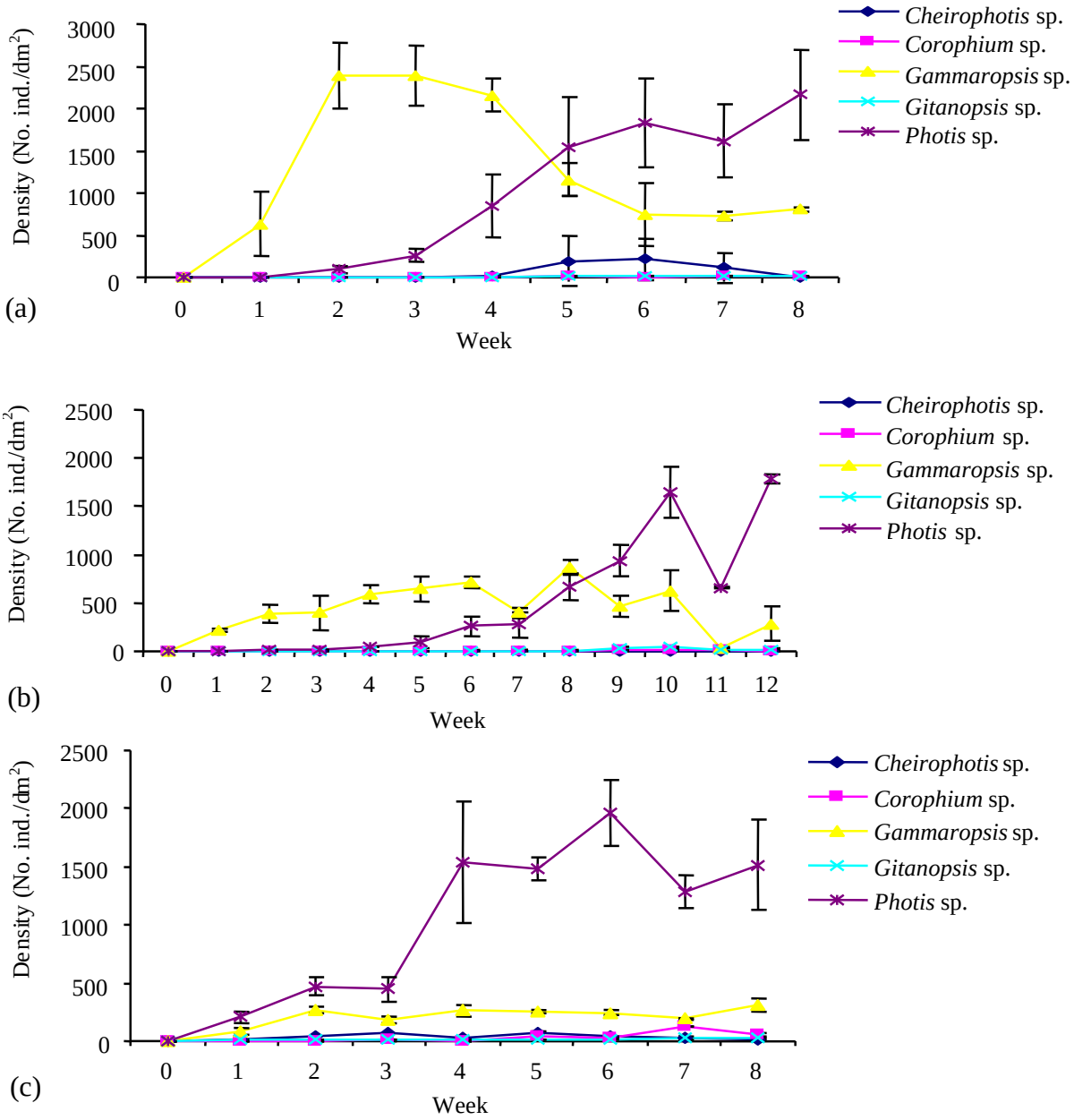


Figure 3.23. Temporal changes in abundance (mean ± SD) of amphipod species on net panels placed inside the pellet cages in a fish farm at (a) Jaha (dry season), (b) Jaha (wet season) and (c) Sangga Besar (dry season).

although the difference was insignificantly ($P > 0.05$). In Sangga Besar, mean density of 36.97 ind./dm^2 was significantly ($P < 0.05$) lower than that in trash-fish feed given net-cages.

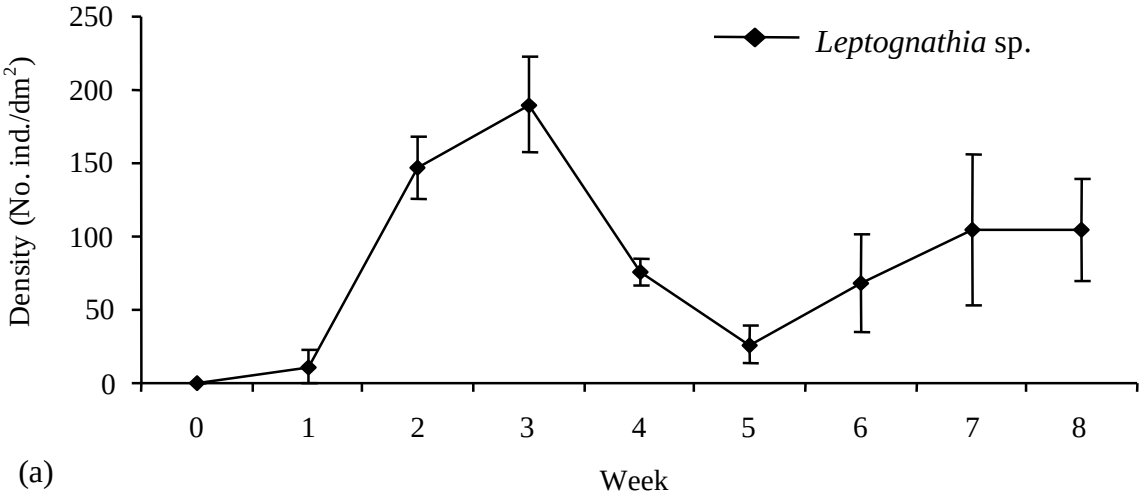
During the dry season at Jaha, weekly development trends and rates of *Leptognathia* sp. on net panels placed inside the net-cages given pellet feed (Figure 3.24a) were quite similar to that in net-cages given trash-fish feed (see Figure 3.9a). However, maximum density of 189.98 ind./dm^2 obtained on the 3rd week was significantly ($P < 0.05$) higher than in net-cages given trash-fish feed. In the wet season (Figure 3.24b), development rates were relatively higher than in net-cages given the trash-fish feed (see Figure 3.16). A maximum density of 81.99 ind./dm^2 on 5th week was significantly ($P < 0.05$) higher than in net-cages given trash-fish feed.

In Sangga Besar, the weekly development rates of *Leptognathia* sp. on net panels placed inside the net-cages given pellet feed (Figure 3.24c) were much slower than in net-cages given trash-fish feed (see Figure 3.9b). The maximum density of 66.99 ind./dm^2 on the 7th week was significantly ($P < 0.05$) lower than that in net-cages given trash-fish feed.

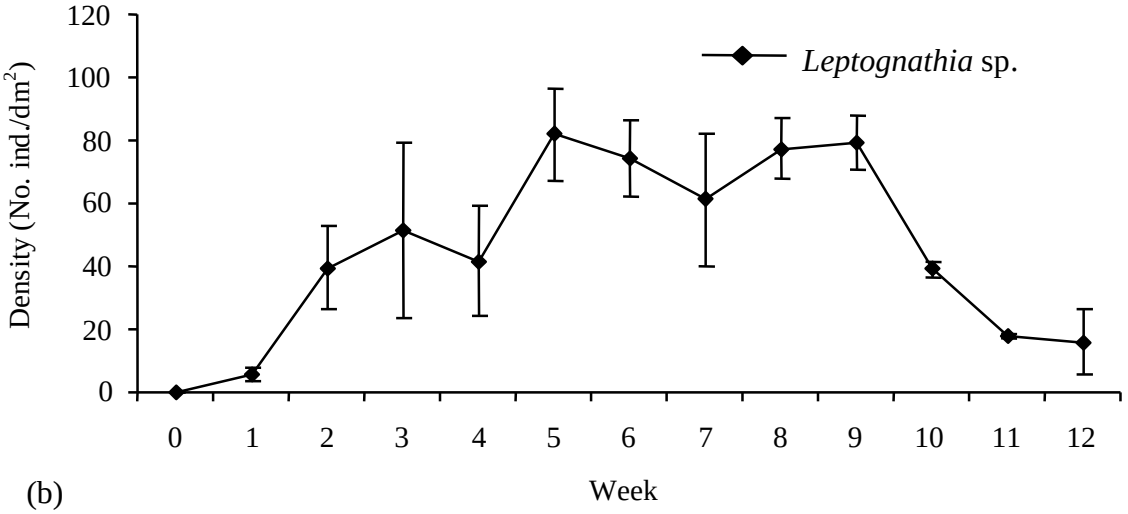
c. Nematoda

Mean density of nematode on net panels placed inside the net-cages given pellet during the dry (41.99 ind./dm^2) and wet (84.76 ind./dm^2) season in Jaha was not significantly ($P > 0.05$) different to that in trash-fish feed given net-cages. However, in Sangga Besar density of 22.86 ind./dm^2 was significantly ($P < 0.01$) lower than in trash-fish cages.

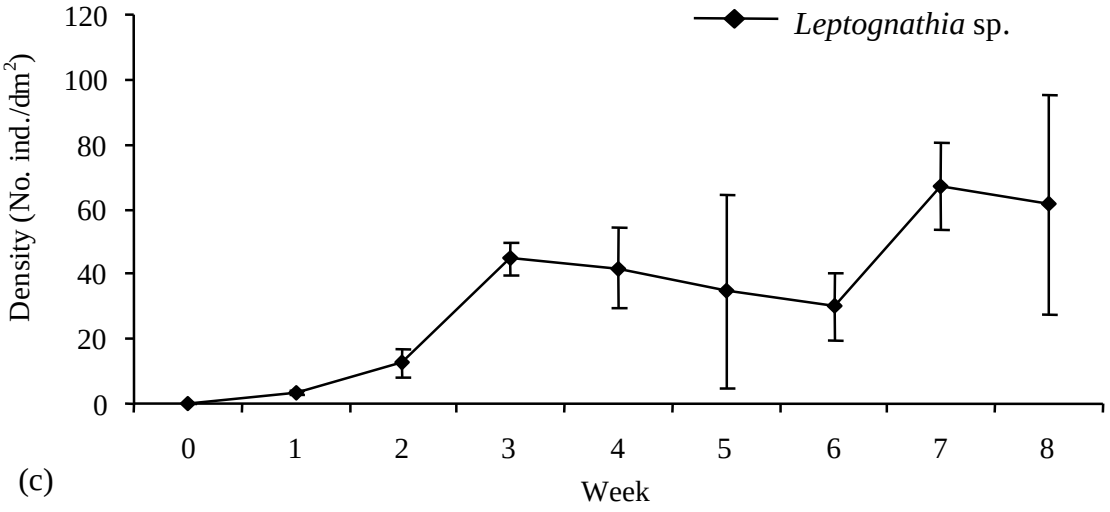
In the dry season at Jaha, weekly development trends of nematode on net panels placed inside the net-cages given pellet feed (Figure 3.25a) were quite similar to that in net-cages given trash-fish feed (see Figure 3.10a). A maximum density of 153.71



(a)



(b)



(c)

Figure 3.24. Temporal changes in abundance (mean ± SD) of *Leptognathia* sp. on net panels placed inside the net-cages given pellet feed in a fish farm at (a) Jaha (dry season), (b) Jaha (wet season) and (c) Sangga Besar (dry season).

ind./dm^2 was obtained at the 2nd week. In the wet season, development trend in pellet cages (Figure 3.25b) was fluctuated, similar to that in net-cages given trash-fish feed (see Figure 3.17). The maximum density of 223.77 ind./dm^2 was achieved at the 5th week.

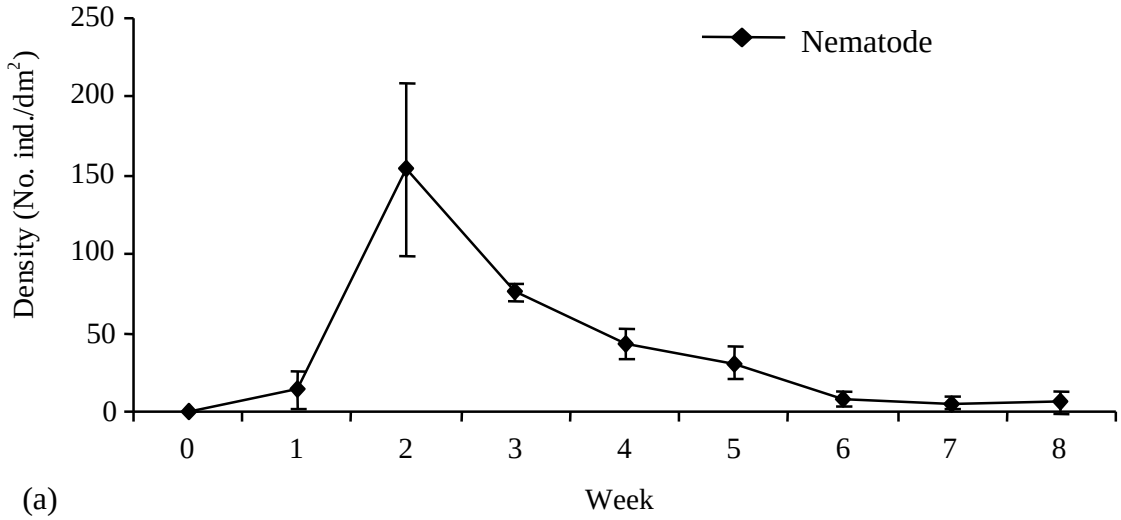
In Sangga Besar, weekly development trend and rates were slightly slower in pellet given net-cages (Figure 3.25c) compared to that in trash-fish feed given net-cages (see Figure 3.10b). The maximum density of 117.01 ind./dm^2 at the 2nd week was much lower than that in trash-fish cages.

d. Copepoda

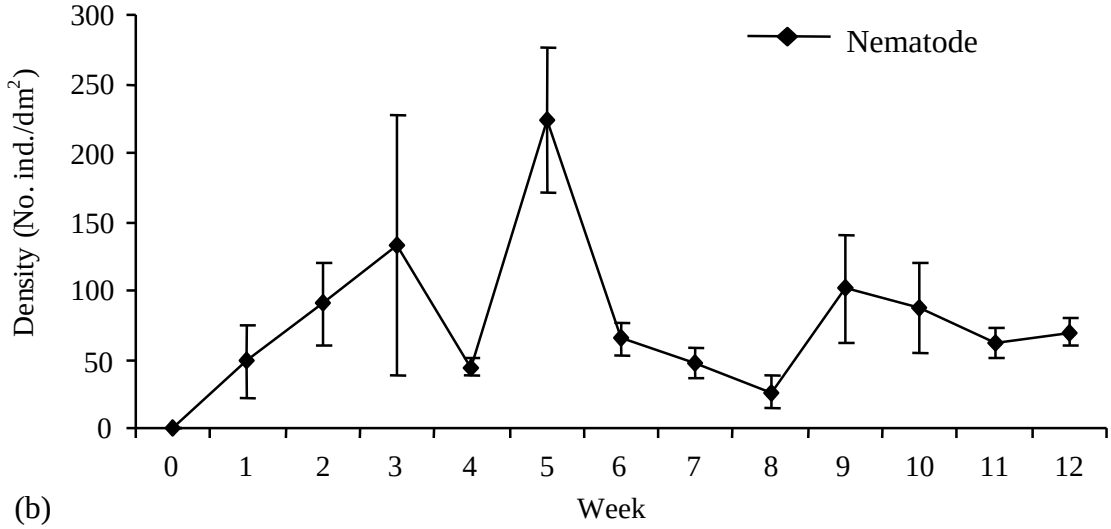
During the dry season at Jaha, mean total copepod density of 14.78 ind./dm^2 on net panels placed inside the net-cages given pellet feed was not significantly ($P > 0.05$) different to that in net-cages given trash-fish feed. However in the wet season, mean density of 35.23 ind./dm^2 was significantly ($P < 0.05$) higher than that in net-cages given trash-fish feed. In Sangga Besar, total mean density of 49.34 ind./dm^2 was significantly ($P < 0.05$) lower than that in net-cages given trash-fish feed.

During the dry season at Jaha, weekly development rates of copepods on net panels placed inside the net-cages given pellet feed (Figure 3.26a) were slightly higher than that in net-cages given trash-fish feed (see Figure 3.11a). The maximum density of 63.37 ind./dm^2 was obtained at the 2nd week. In the wet season, weekly development rates were much higher in pellet cages (Figure 3.26b) than that in trash-fish cages (see Figure 3.18), in particularly the consecutive 5th to 10th week. The maximum density of186.45 ind./dm^2 was obtained at the 9th weeks.

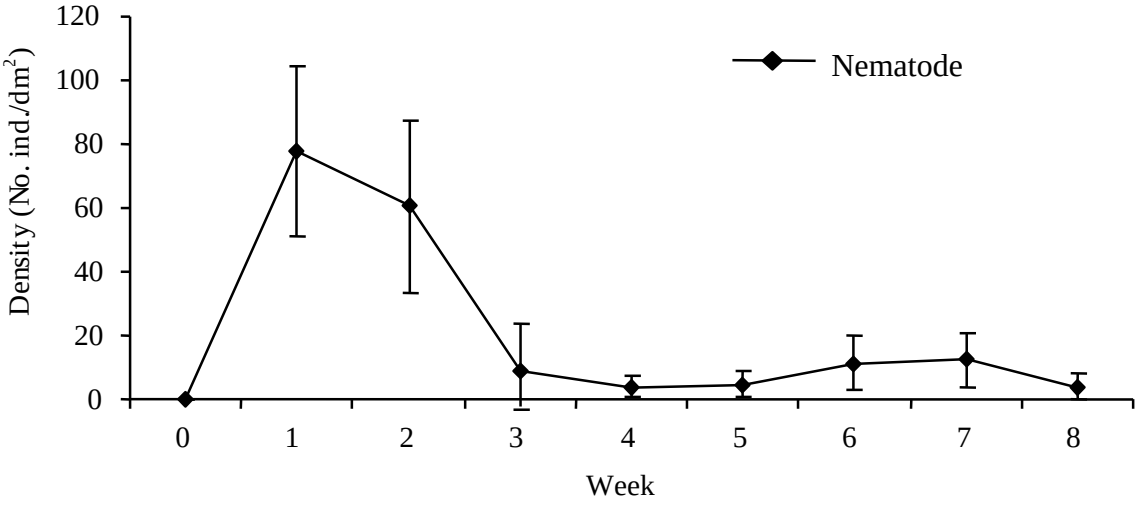
In Sangga Besar, weekly development rates of copepods in pellet cages (Figure 3.26c) were slightly slower than in trash-fish cages (see Figure 3.11b). The maximum density of 115.91 ind./dm^2 at the 2nd week, was much lower than that in net-cages given



(a)



(b)



(c)

Figure 3.25. Temporal changes in abundance (mean ± SD) of nematode on net panels placed inside the net-cages given pellet feed in a fish farm at (a) Jaha (dry season), (b) Jaha (wet season) and (c) Sangga Besar (dry season).

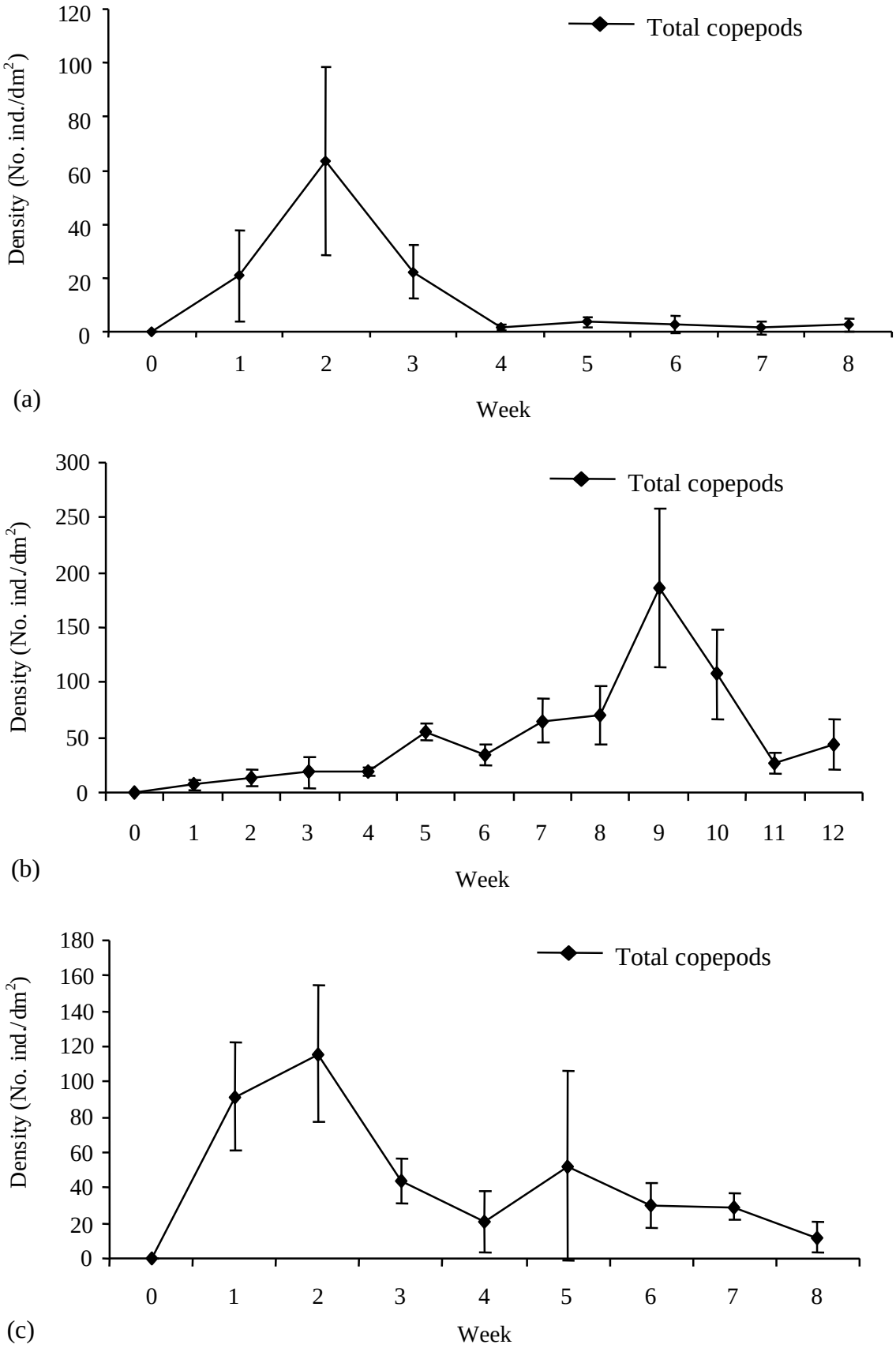


Figure 3.26. Temporal changes in abundance (mean ± SD) of copepods on net panels placed inside the net-cages given pellet feed in a fish farm at (a) Jaha (dry season), (b) Jaha (wet season) and (c) Sangga Besar (dry season).

trash-fish feed.

E. acutifrons was the dominant copepod on net panels placed inside the net-cages given pellet feed (Table 3.8), similar to that in net-cages given trash-fish feed in both seasons in Jaha. The mean density of 13.96 ind./dm^2 and 32.97 ind./dm^2 during the dry and wet season respectively was not significantly different to that in net-cages given trash-fish feed for both dry (see Table 3.4) and wet (see Table 3.6) seasons. In Sangga Besar, mean density (39.29 ind./dm^2) of *E. acutifrons* was significantly ($P < 0.001$) lower than that in net-cages given trash-fish feed (see Table 3.4).

During the dry season in Jaha, weekly development rates of *E. acutifrons* on net panels placed inside the net-cages given pellet (Figure 3.27a) were relatively similar to that in trash-fish cages (see Figure 3.12a). A maximum density of 58.49 ind./dm^2 was obtained at the 2nd week. However, in the wet season (Figure 3.27b) weekly development rates were relatively higher than in net-cages given trash-fish feed (see Figure 3.19a). A maximum density of 180.43 ind./dm^2 at the 9th week was significantly higher than that in net-cages given trash-fish feed.

In Sangga Besar, weekly development rates of *E. acutifrons* on net panels placed inside the net-cages given pellet feed (Figure 3.27c) was slightly slower than in trash-fish cages (see Figure 3.12b). The maximum density of 81.87 ind./dm^2 at the 2nd week, was significantly ($P < 0.05$) lower than in net-cages given trash-fish feed.

e. Polychaeta and Others

The density of polychaete and other non-dominance species was not significantly ($P > 0.05$) different between pellet and trash-fish feed for both season and estuary.

Table 3.8. Mean density of copepod species over eight weeks of colonization on net panels placed inside the net-cages given pellet feed in a fish farm at Jaha (dry and wet season), and Sangga Besar (dry season only). Standard deviation (SD) in parentheses.

	Jaha (dry season) (No. ind./dm^2)	Jaha (wet season) (No. ind./dm^2)	Sangga Besar (dry season) (No. ind./dm^2)
A.. pasifica	0.14 (0.21)	0.05 (0.09)	0 (0)
Copepod larvae	0.38 (0.53)	0.07 (0.09)	0.02 (0.04)
E. acutifrons	13.96 (8.00)	32.97 (9.35)	39.29 (15.56)
Microcalanus sp. 1	0.08 (0.10)	0.09 (0.16)	0 (0)
Oithona simplex	0.04 (0.07)	0.00 (0.00)	0 (0)
Oncaea sp.	0.02 (0.04)	0.21 (0.26)	0 (0)
Paracalanus sp. 1	0.04 (0.06)	0.11 (0.15)	2.74 (2.54)
Tigriopus sp.	0.12 (0.16)	1.50 (1.31)	0.08 (0.14)
Paracalanus sp. 2	0.00 (0.00)	0.17 (0.23)	6.98 (4.03)
Microcalanus sp. 2	0.00 (0.00)	0.02 (0.04)	0 (0)
Saphierella-like copepodid	0.00 (0.00)	0.03 (0.05)	0 (0)

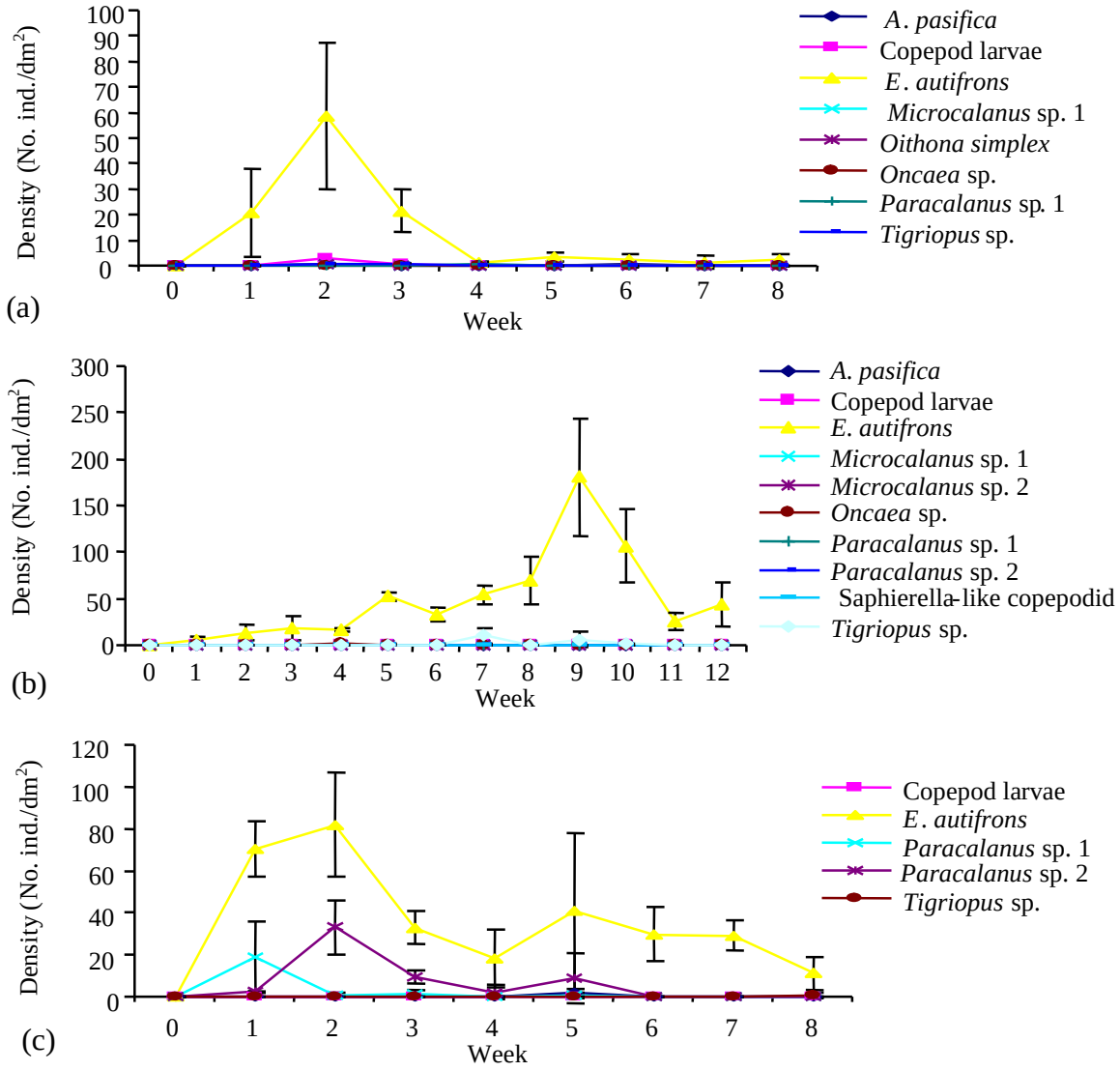


Figure 3.27. Temporal changes in abundance (mean ± SD) of copepod species on net panels placed inside the net-cages given pellet feed in a fish farm at (a) Jaha (dry season), (b) Jaha (wet season) and (c) Sangga Besar (dry season only).

3.2.7. Macrofouling Community of Net Panels Suspended Outside Net-Cages (Dry and Wet Season)

3.2.7.1. Species Composition of Sessile and Non-Sessile Associates

During the dry season at Jaha, sessile macrofouling community on net panels placed outside the net-cages (without any fish feed input i.e. pellet or trash-fish feed) were similar to that inside net-cages given pellet or trash-fish feed. However, in the wet season *B. amphitrite* and *Lyngbya* sp. were not present. In Sangga Besar, *Lyngbya* sp. was not encountered, however the other sessile macrofouling species were similar to that inside net-cages given feed.

During the dry season at Jaha, the number of non-sessile species outside the net-cages was 25. *Microcalanus* sp.1, *Microcalanus* sp. 2, *Paracalanus* sp. 2, *Paracalanus* sp.1 and Terebellidae were encountered but not in net-cages given feed. In the wet season, number was 27. In Sangga Besar, total of 24 non-sessile species were enumerated outside the net-cages, but the dominant species were similar to that inside the net-cages given feed.

3.2.7.2. Depth Distribution and Percentage Cover of Sessile Macrofouling Organisms

During the dry season at Jaha, depth distribution of sessile macrofoulers on net panel placed outside the net-cages (Figure 3.28a) was similar to that inside net-cages given trash-fish (see Figure 3.5a) or pellet (see Figure 3.20a) feed. The mean percentage covers of *Plumularia* sp. and *Lyngbya* sp. at all depth stratums were significantly ($P < 0.05$) higher than in pellet or trash-fish cages. However, percentages cover of *Polysiphonia* sp., anthozoans, *X. mangle* and *B. amphitrite* at all depth strata were significantly ($P < 0.05$) lower compared to that inside the net-cages given feed.

In the wet season, mean percentage cover of *Plumularia* sp. at all depth strata was significantly ($P < 0.05$) higher (Figure 3.28b) than that inside the net-cages given feed

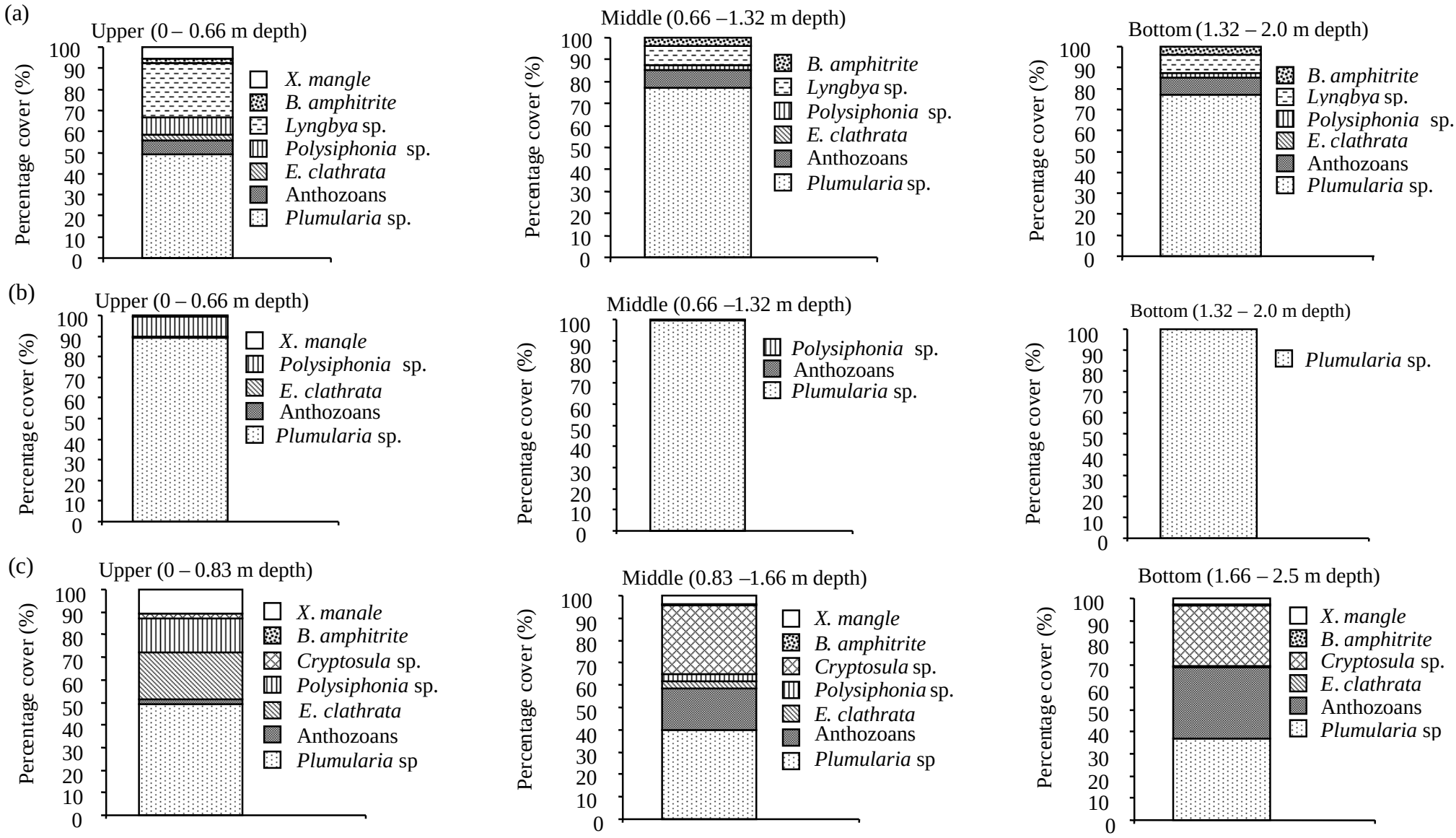


Figure 3.28. Depth distribution and percentage cover of sessile macrofouling organisms (upper, middle & bottom strata) over eight weeks of colonization on net panels placed outside the net-cages in a fish farm at (a) Jaha (dry season), (b) Jaha (wet season) and (c) Sangga Besar (dry season).

i.e. trash-fish (see Figure 3.13a) or pellet (see Figure 3.20b) feed. However, percentage cover of *Polysiphonia* sp., anthozoans and *X. mangle* at the middle and bottom stratum was significantly ($P < 0.05$) much lower than that inside the net-cages given feed.

In Sangga Besar, depth distribution of sessile macrofoulers on net panels placed outside the net-cages (Figure 3.28c) was mush similar to that in trash-fish (see Figure 3.5b) or pellet (see Figure 3.20c) cages. Their percentage cover was not significantly ($P > 0.05$) different to that inside net-cages given feed (see Appendix 4).

3.2.7.3. Temporal Change in Species Composition and Percentage Cover of Sessile Macrofouling Organisms

In the dry season at Jaha, sessile macrofouling organisms of net panels placed outside the net-cages started with the dominance of *Plumularia* sp. (Figure 3.29a). It developed quicker than in net panels placed inside the net-cages given trash-fish (see Figure 3.6a) or pellet (see Figure 3.21a) feed. However, other species such as *Lyngbya* sp., anthozoans, *B. amphitrite* and *X. mangle* developed slowly compared to that on net panels given trash-fish or pellet feed.

In the wet season at Jaha, *Plumularia* sp. on net panel placed outside net-cages almost completely dominated (100%) net community until the completion of the experiment (Figure 3.29b). Only *Polysiphonia* sp. and *X. mangle* were also present at the upper stratum but their percentage covers were significantly lower than inside the net-cages, whether given trash-fish (see Figure 3.13b) or pellet (see Figure 3.21b) feed. Development rates of anthozoans and *E. clathrata* were relatively much slower and sporadic colonies were found at the upper and middle strata only.

The weekly development of sessile macrofouling organism on net panels placed outside the net-cages (Figure 3.29c) at Sangga Besar was relatively fluctuated similar to that in trash-fish (see Figure 3.6b) or pellet (see Figure 3.21c) cages. The *Plumularia*

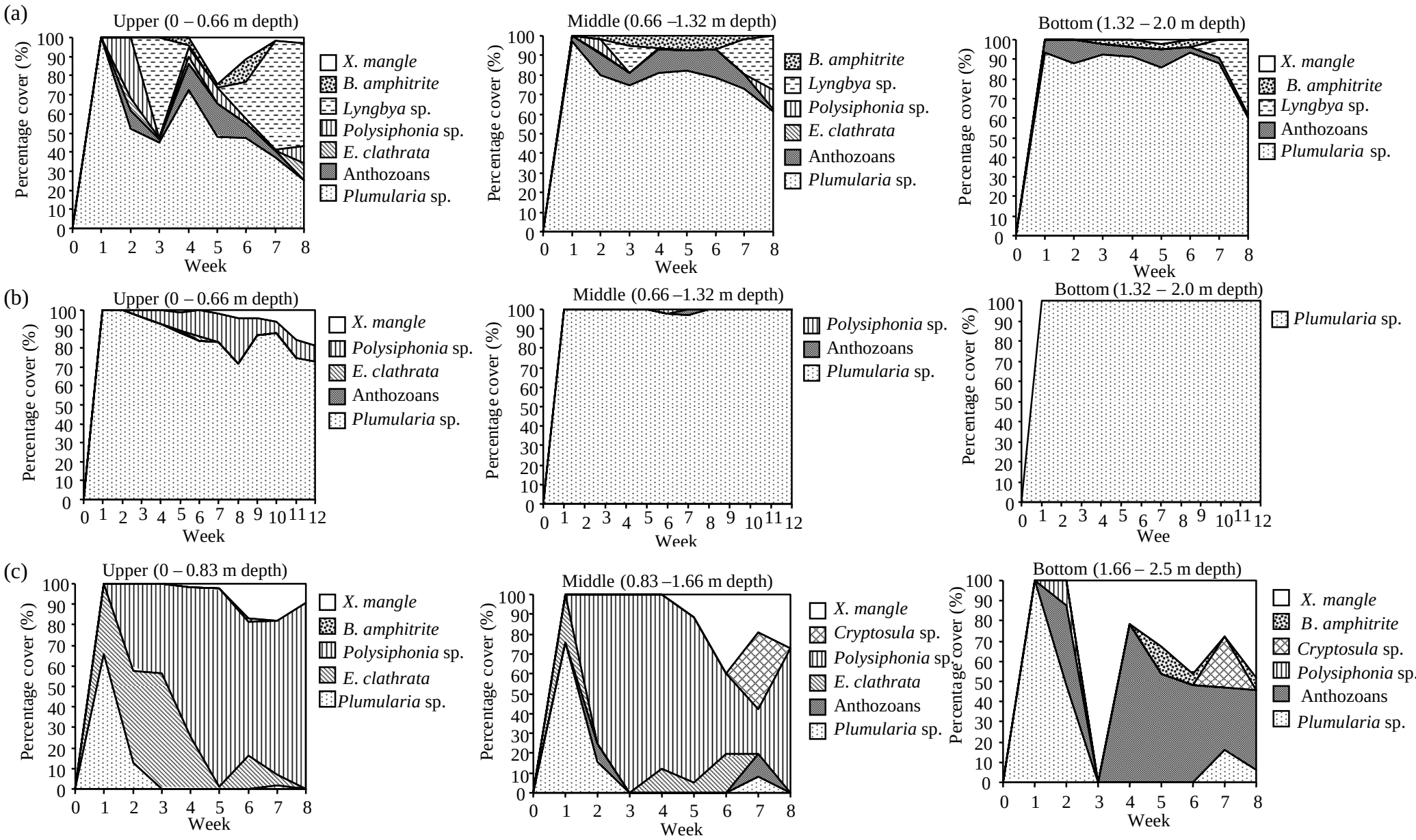


Figure 3.29. Temporal changes in species composition and percentage cover of sessile macrofouling organisms at the upper, middle and bottom strata of net panels placed outside the net-cages in a fish farm at Jaha (dry season) (a), Jaha (wet season) (b) and Sangga Besar (dry season) (c).

sp., *E. clathrata* and anthozoans were among the important early colonizers, which dominated the net panels as early at 1st week. Competition among *Plumularia* sp., anthozoans and *Cryptosula* sp. occurred at the middle and bottom stratum, similar to that in net-cages given feed.

3.2.7.4. Temporal Change in Species Composition and Abundance of Non-Sessile Associates

a. Amphipoda

Total density of amphipods on net panels placed outside the net-cages during the dry (323.77 ind./dm^2) and wet (168.02 ind./dm^2) season at Jaha was significantly ($P < 0.001$) lower than that inside the net-cages given fish feed. In Sangga Besar, total mean density of 617.82 ind./dm^2 was not significantly ($P > 0.05$) different to that in net-cages given feed (see Appendix 5).

The weekly development rate of the amphipod population outside the net-cage was much slower than inside the net-cages given trash-fish or pellet feed for both dry and wet seasons at Jaha. During the dry season (Figure 3.30a), density gradually increased to a maximum 560.22 ind./dm^2 on the 3rd week. This density was almost four times lower than inside the net-cages given feed whether trash-fish (see Figure 3.7a) or pellet (see Figure 3.22a) feed. In the wet season (Figure 3.30b), maximum density of 349.95 ind./dm^2 was obtained on 7th week which almost two times lower than that in the net-cages given trash-fish (see Figure 3.14) or pellet (Figure 3.22b) feed.

In Sangga Besar, development rates of the amphipod population outside the net-cages (Figure 3.30c) were much slower than inside the net-cages given pellet (see Figure 3.22c), although it relatively higher than in trash-fish feed (see Figure 3.7b) given net- cages. The maximum density of 854.72 ind./dm^2 was obtained on 5th week.

The *Gammaropsis* sp. and *Photis* sp. was an important non-sessile macrofoulers of

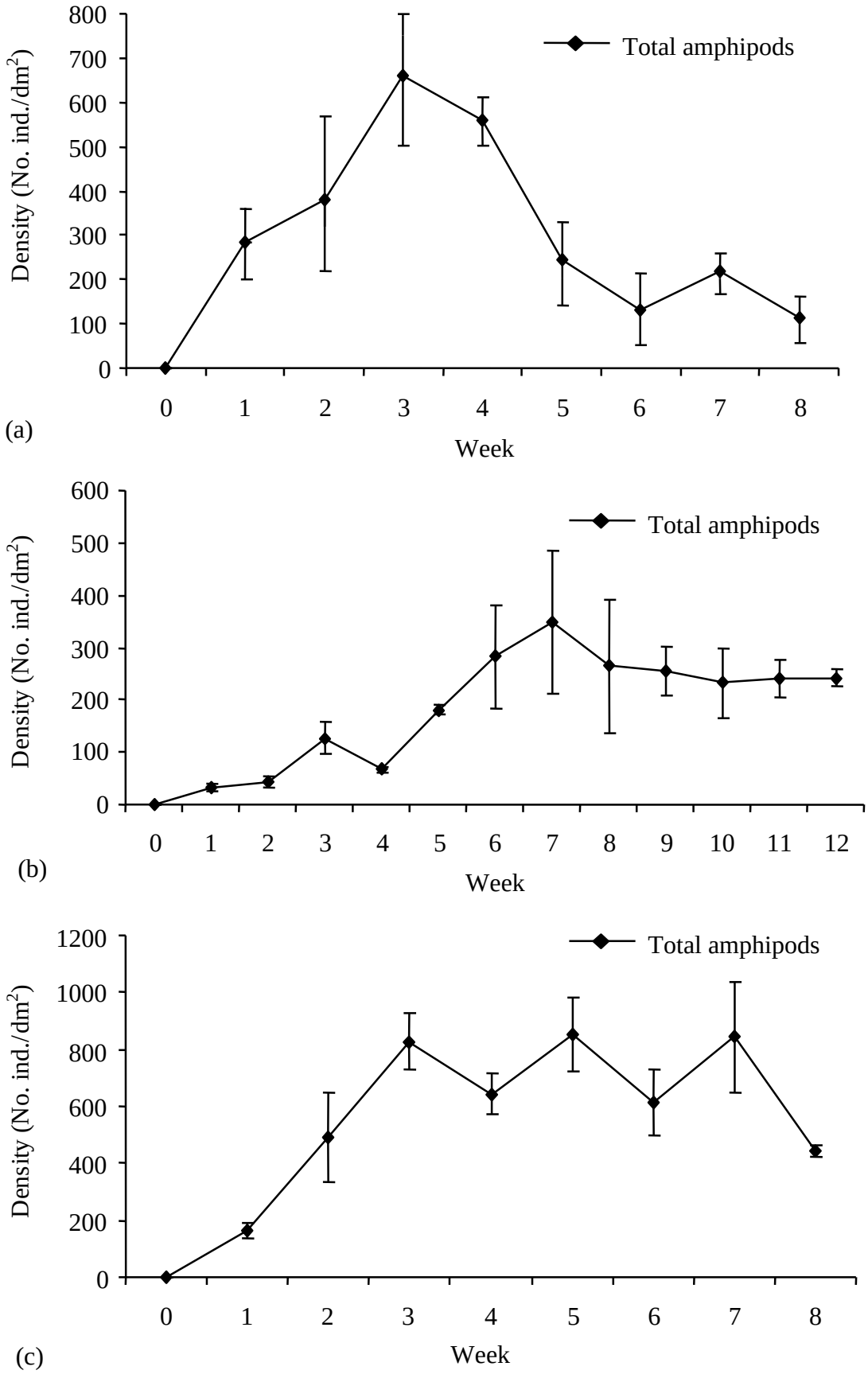


Figure 3.30. Temporal changes in the abundance (mean ± SD) of amphipods on a net panels placed outside the net-cages in a fish farm at (a) Jaha (dry season), (b) Jaha (wet season) and (c) Sangga Besar (dry season).

net panels placed outside the net-cages (Table 3.9) similar to that inside net-cages given trash-fish during the dry (see Table 3.3) and wet (see Table 3.5) season, or given pellet feed during the dry and wet (see Table 3.7) season at Jaha. However, their mean density was significantly ($P < 0.05$) much lower. During the dry season, mean densities of *Gammaropsis* sp. and *Photis* sp. was 298.99 ind./dm^2 and 19.34 ind./dm^2 respectively, while in the wet season these were 158.73 ind./dm^2 and 5.93 ind./dm^2 respectively.

In Sangga Besar, mean density of *Photis* sp. (302.22 ind./dm^2) on net panel placed outside the net-cages (see Table 3.9) was significantly lower than in pellet cages (see Table 3.7) but this was not significantly different to that in trash-fish feed (see Table 3.3) given net-cages. Mean density (222.14 ind./dm^2) of *Gammaropsis* sp. was not significantly different to that in feed given net-cages. Population density of other amphipods species was relatively low similar to that in net-cages given fish feed in both season and estuary.

The weekly development rate of *Gammaropsis* sp. on net panels placed outside the net-cages was much slower than that in the net-cages given trash-fish (see Figure 3.8a, dry season; Figure 3.15, wet season) or pellet (see Figure 3.23a, dry season; Figure 3.23b, wet season) feed at Jaha. During the dry season (Figure 3.31a), maximum density of 634.76 ind./dm^2 was obtained on the 3rd week, while in the wet season (Figure 3.31b), density gradually increased to a maximum 279.74 ind./dm^2 on the 7th week. Weekly development of *Photis* sp. was much slower than that inside the net-cages given feed during the dry and wet season at Jaha. There were appeared to be no competitions between *Gammaropsis* sp. and *Photis* sp., although populations by *Gammaropsis* sp. reduced at the near end of the experiment.

In Sangga Besar, weekly development rates of Photis sp. and Gammaropsis sp. outside the net-cages (Figure 3.31c) was relatively slower than that inside the net-cages

Table 3.9. Mean density of amphipod species over eight weeks of colonization on net panels placed outside the net-cages in a fish farm at Jaha (dry and wet season) and Sangga Besar (dry season only). Standard deviation (SD) in parentheses.

	Jaha (dry season) (No. ind./dm^2)	Jaha (wet season) (No. ind./dm^2)	Sangga Besar (dry season) (No. ind./dm^2)
Cheirophotis sp.	3.43 (1.94)	0.16 (0.28)	68.03 (12.38)
Corophium sp.	0.67 (0.59)	0.51 (0.46)	11.20 (3.45)
Gammaropsis sp.	298.99 (79.32)	158.73 (55.15)	222.14 (49.26)
Gitanopsis sp.	1.22 (1.08)	3.92 (2.83)	12.80 (3.11)
Photis sp.	19.34 (15.58)	5.93 (5.75)	302.22 (34.27)

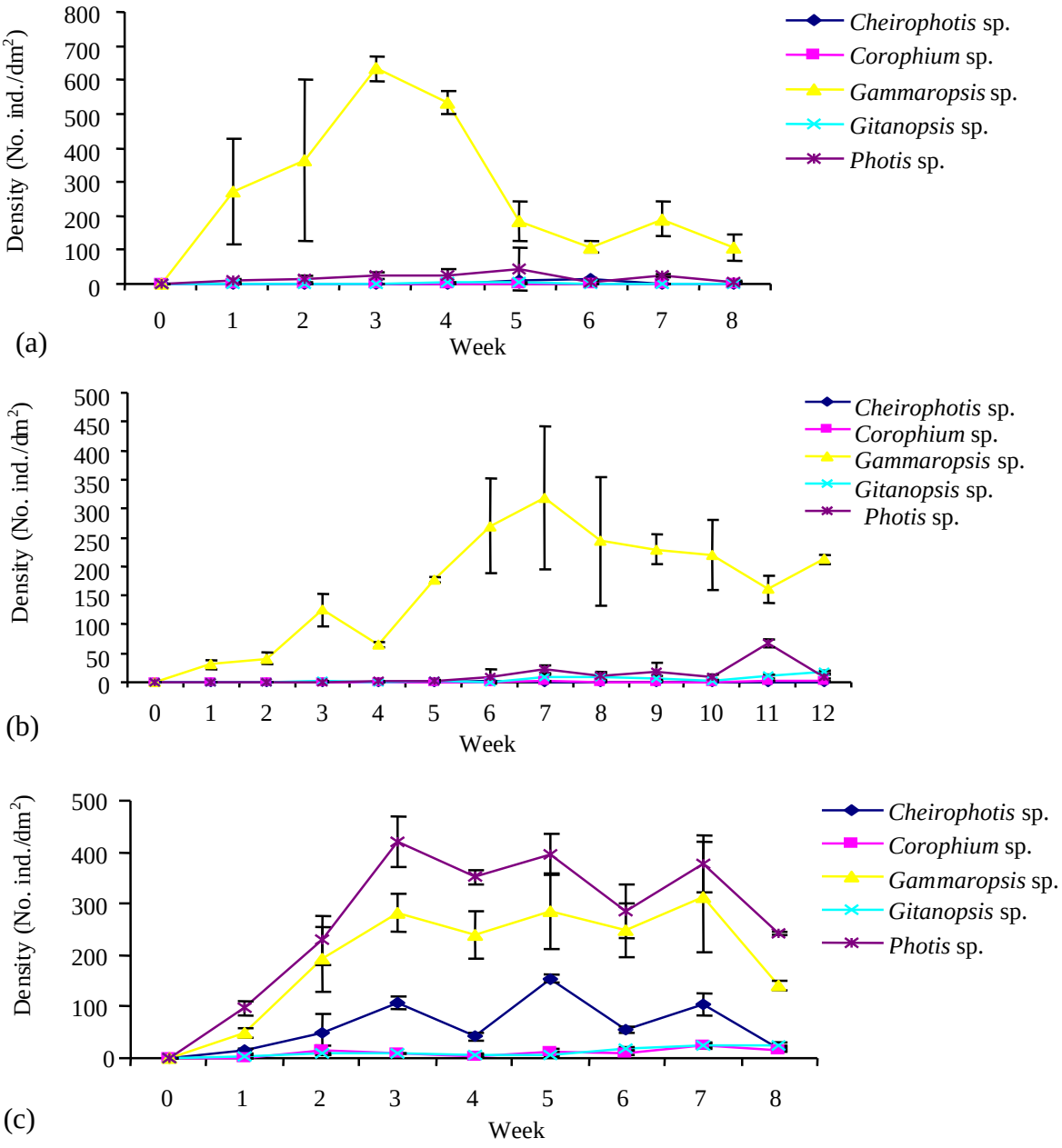


Figure 3.31. Temporal changes in abundance (mean ± SD) of amphipod species on net panels placed outside the net-cages in a fish farm at (a) Jaha (dry season), (b) Jaha (wet season) and (c) Sangga Besar (dry season) River estuary.

Given trash-fish (see Figure 3.8b) or pellet (see Figure 3.23c) feed. Maximum density of 420.33 ind./dm^2 for Photis sp was achieved at the 2nd week while maximum of 313.55 ind./dm^2 achieved at the 7th week for Gammaropsis sp..

Development rates of other amphipods species were generally slower similar to that in net-cages given feed (trash fish or pellet).

b. Tanaidacea

Mean density of *Leptognathia* sp. outside the net-cages during the dry (10.24 ind./dm^2) and wet (5.97 ind./dm^2) seasons was significantly ($P < 0.05$) much lower than in the net-cages given pellet and trash-fish feed at Jaha. In Sangga Besar, mean density of 32.59 ind./dm^2 was almost similar to that inside the net-cages given pellet feed. However, this was significantly ($P < 0.05$) much lower than that in trash-fish cages.

During the dry season at Jaha, the weekly development rates of *Leptognathia* sp. was slower outside the net-cages (Figure 3.32a) compare to that inside net-cages given trash-fish (see Figure 3.9a) or pellet (see Figure 3.24a) feed. A maximum density of 15.72 ind./dm^2 obtained on the 3rd week. Development rates were even slower during the wet season (Figure 3.32b) compared to trash-fish (see Figure 3.16) and pellet (see Figure 3.24b) feed respectively, maximum density of 36.63 ind./dm^2 obtained at the 11th week.

In Sangga Besar, weekly development rates were relatively slower compare to that inside the net-cages given feed. The maximum density of 83.29 ind./dm^2 obtained at the 7th week was significantly ($P < 0.05$) (Figure 3.32c), higher than in net-cages given pellet feed (see Figure 3.24c) but relatively lower compare to that inside the net-cages given trash-fish feed (see Figure 3.9b).

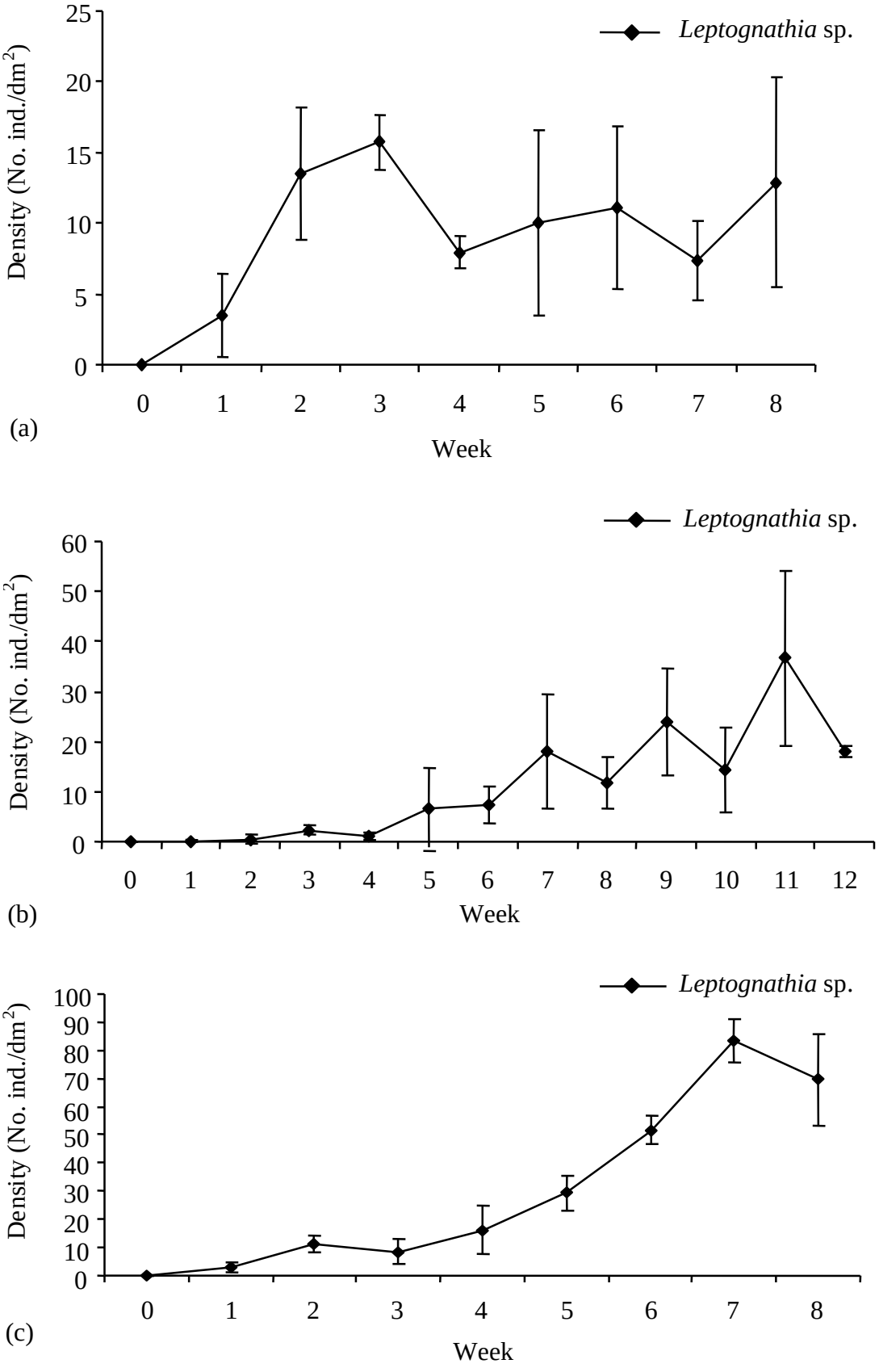


Figure 3.32. Temporal changes in abundance (mean ± SD) of *Leptognathia* sp. on net panels placed outside the net-cages in a fish farm at (b) Jaha (dry season), (c) Jaha (wet season) and (d) Sangga Besar (dry season) River estuary.

c. Nematoda

During the dry season at Jaha, mean density (82.20 ind./dm^2) of nematode outside the net-cages was significantly higher than in net-cages given pellet or trash-fish feed. In the wet season, mean density was higher at 62.23 ind./dm^2, however this was not significantly ($P > 0.05$) different to that in net-cages given feed. In Sangga Besar, mean density of 62.15 ind./dm^2 was significantly higher than in pellet given net-cages (see Figure 3.26a), although this was not significantly different to that in trash-fish given net-cages.

In the dry season at Jaha, weekly development rates of nematode were much higher outside the net-cage (Figure 3.33a) than that in net-cages given trash-fish (see Figure 3.10a) or pellet (see Figure 3.25a) feed. The maximum density of 203.85 ind./dm^2 was obtained at the 2^{nd} week. In the wet season (Figure 3.33b), development trend was fluctuated similar to that in net-cages given trash-fish (see Figure 3.17) or pellet (see Figure 3.25b) feed. The maximum density of 134.17 ind./dm^2 was obtained at the 11^{th} week.

In Sangga Besar, the development trends of nematode outside the net-cages (Figure 3.33c) were quite similar to that in net-cages given trash-fish (see Figure 3.10b) and pellet feed (see Figure 3.25c). The maximum density of 117.01 ind./dm^2 obtained at the 2^{nd} week.

d. Copepoda

Total mean density of copepods outside the net-cages during the dry (38.05 ind./dm^2) and wet (93.55 ind./dm^2) season at Jaha was significantly ($P < 0.001$) higher than in the net-cages given pellet and trash-fish feed respectively. In Sangga Besar, total mean density (152.16 ind./dm^2) was significantly higher than inside the net-cages given pellet feed, although it was relatively similar to that in trash-fish cages.

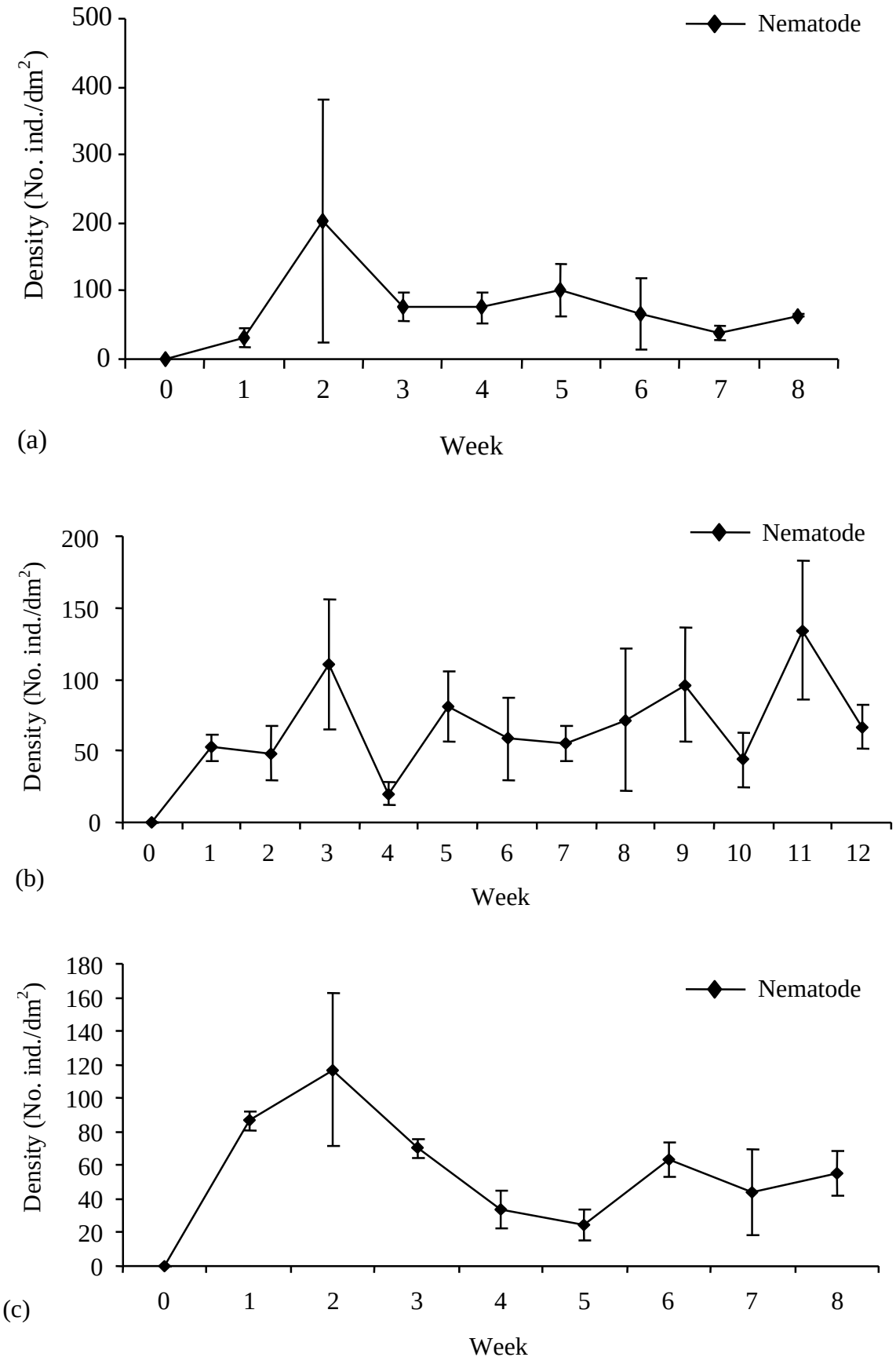


Figure 3.33. Temporal changes in abundance (mean ± SD) of nematode on net panels placed outside the net-cages in a fish farm at (b) Jaha (dry season), (b) Jaha (wet season) and (c) Sangga Besar (dry season) River estuary.

During the dry season at Jaha, weekly development rates of copepods population outside the net-cages (Figure 3.34a) were relatively higher than that in trash-fish (see Figure 3.11a) or pellet (see Figure 3.26a) feed given net-cages. The maximum density of 77.53 ind./dm^2 was obtained at the 3rd week. In the wet season (Figure 3.34b), development rates were even higher than that inside the net-cages given feed trash-fish (see Figure 3.18) or pellet feed (see Figure 3.26b). The maximum density of 164.54 ind./dm^2 obtained at the 9th week. In Sangga Besar, the increment rates of copepods population outside the net-cages (Figure 3.34c) were more variable, however it relatively higher than that in net-cages given trash-fish (see Figure 3.11b) or pellet (see Figure 3.26c) feed. The maximum density of 253.01 ind./dm^2 was obtained at the 6th weeks.

E. acutifrons was the dominant copepods on net panels placed outside the net-cages, similar to that in net-cages given feed. In Jaha, mean density of 27.75 ind./dm^2 and 60.78 ind./dm^2 during the dry and wet season respectively (Table 3.10) was significantly ($P < 0.05$) higher than that in net-cages given trash-fish (see Table 3.4, dry season; Table 3.6, wet season) or pellet (see Table 3.8, dry & wet season) feed respectively. Mean density of other copepods species was generally lower and mainly found outside the net-cages. In Sangga Besar, mean density (119.07 ind./dm^2) of *E. acutifrons* was significantly higher ($P < 0.001$) (see Table 3.10) than in pellet (see Table 3.8) given net- cages but relatively no different to that in trash-fish (see Table 3.4) net-cages. *Paracalanus* sp. 2 was among the important copepod outside the net-cages at Sangga Besar. Mean density was significantly ($P < 0.05$) higher than inside the net-cages given feed.

During the dry season in Jaha, weekly development rates of *E. acutifrons* were much higher outside the net-cages (Figure 3.35a) than inside the net-cages given trash-fish (see Figure 3.12a) or pellet (see Figure 3.23a) feed. Density increased rapidly to a

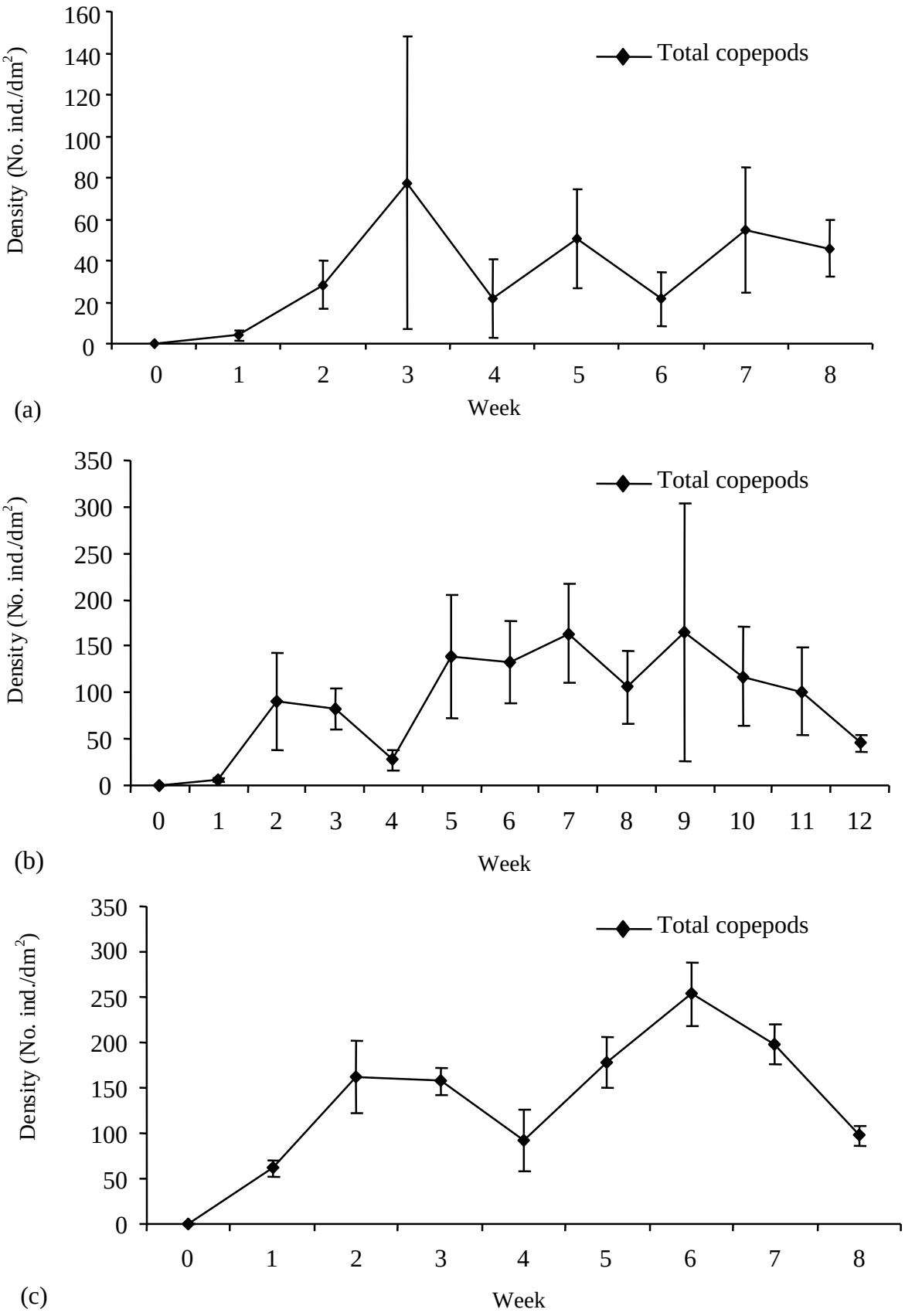


Figure 3.34. Temporal changes in abundance (mean ± SD) of copepods on net panels placed outside the net-cages in a fish farm at (a) Jaha (dry season), (b) Jaha (wet season) and (c) Sangga Besar (dry season).

Table 3.10. Mean density of copepod species over eight weeks of colonization on net panels placed outside the net-cages in a fish farm at Jaha (dry and we season) and, Sangga Besar (dry season only). Standard deviation in parenthesis.

	Jaha (dry season) (No. ind./dm^2)	Jaha (wet season) (No. ind./dm^2)	Sangga Besar (dry season) (No. ind./dm^2)
A. pasifica	0.37 (0.26)	6.21 (4.00)	0.05 (0.05)
Copepod larvae	1.14 (0.82)	2.21 (1.97)	0.02 (0.04)
E. acutifrons	27.75 (13.96)	60.79 (17.35)	119.08 (15.43)
Microcalanus sp. 1	0.82 (0.92)	1.31 (1.11)	0.00 (0.00)
Microcalanus sp. 2	0.38 (0.61)	4.25 (2.35)	0.09 (0.14)
Oithona simplex	0.10 (0.06)	0.23 (0.40)	0.00 (0.00)
Oncaea sp.	0.01 (0.02)	0.61 (0.73)	0.03 (0.06)
Paracalanus sp. 1	0.78 (1.31)	0.03 (0.06)	3.39 (1.06)
Paracalanus sp. 2	5.95 (4.07)	17.19 (7.59)	28.33 (6.48)
Saphierella-like copepodid	0.00 (0.00)	0.40 (0.34)	0.01 (0.02)
Tigriopus sp.	0.22 (0.25)	0.09 (0.11)	0.69 (1.13)

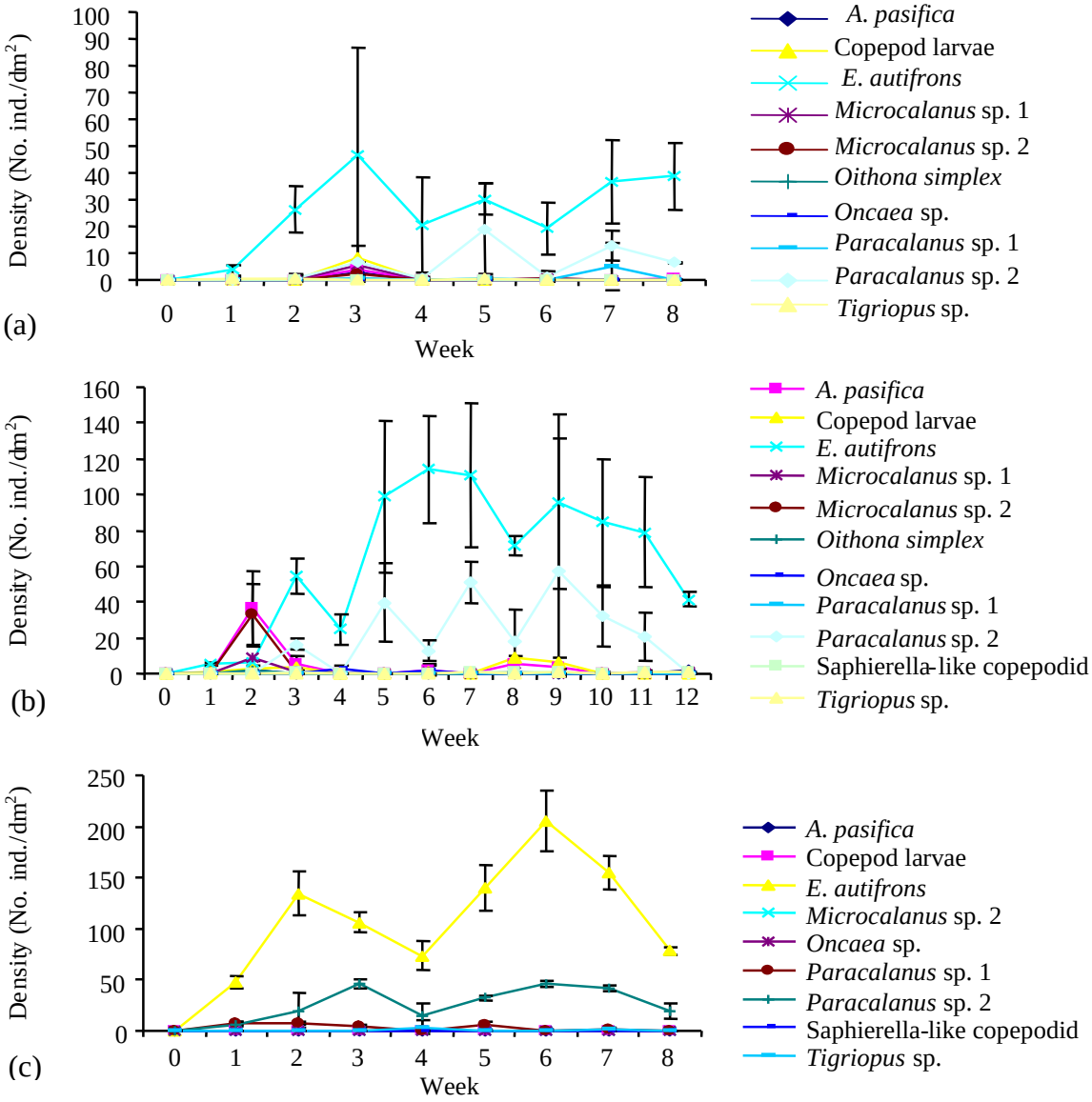


Figure 3.35. Temporal changes in abundance (mean ± SD) of copepod species on net panels placed outside the net-cages in a fish farm at (a) Jaha (dry season), (b) Jaha (wet season) and (c) Sangga Besar (dry season only).

maximum of 46.75 ind./dm^2 at the 3rd week. In the wet season (Figure 3.35b), the development rates were even higher than inside the net-cages given trash-fish (see Figure 3.19) or pellet (see Figure 3.27b) feed. The maximum density of 114.07 ind./dm^2 obtained on the 6th week.

In Sangga Besar, the increment rates of *E. acutifrons* outside the net-cages (Figure 3.35c) was relatively higher than that in net-cages given trash-fish (see Figure 3.12b) or pellet (see Figure 3.27c) feed, however the development trends was more or less similar to that in net-cages given trash-fish feed. The maximum density of 205.46 ind./dm^2 at the 6th week was significantly higher than that in both net-cages given feed.

e. Polychaeta and others

The density of polychaete and other single species on net panels placed outside the et-cages was not significantly ($P > 0.05$) different to that in net-cages given feed in both seasons and estuaries. Their density was relatively lower and present occasionally, similar to the observation inside the net-cages given feed.

3.2.8. Total Wet Biomass of Macrofouling Assemblages

3.2.8.1. Sessile Macrofouling

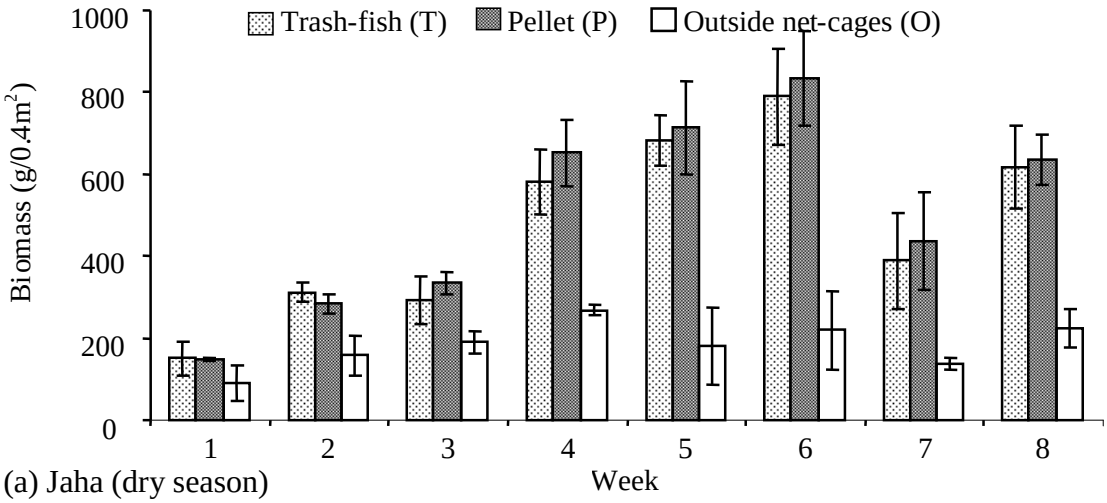
During the dry season at Jaha, mean wet biomass (g per 0.4 m^2 net panel dimension, or g per panel) of sessile macrofouling after eight weeks of development was significantly ($P < 0.05$) higher inside net-cages given pellet (505.02 g) and trash-fish feed (445.29 g) than outside the net-cages (183.4 g). Although the biomass was higher in pellet than in the trash-fish cages, the difference was insignificant ($P > 0.05$). In the dry season, the biomass of sessile macrofoulers inside net-cages given feed had rapidly increased by the 5th – 6th week, reaching maximum wet biomass of around 700 – 850 g, compared to less than 300 g outside the net-cages (Figure 3.36a) (Appendix 6a).

The eight-weeks comparison between the dry and wet season at Jaha, indicate that mean biomass (g per 0.4 m^2 net panel dimension) of sessile macrofoulers in pellet (213.05 g) and trash-fish (257.85 g) feed during the wet season was significantly ($P < 0.01$) much lower than during the dry season. However, without fish culture (outside net-cages), there were no significant ($P > 0.05$) difference between dry and wet (161.85 g) seasons. The development rates were generally slower during the wet season (Figure 3.36b). Maximum biomass of around 600 – 1000 g were obtained on the 11th week in both feeding treatments compared to less than 250 g outside the net-cages. These rates were generally slower than during the dry season.

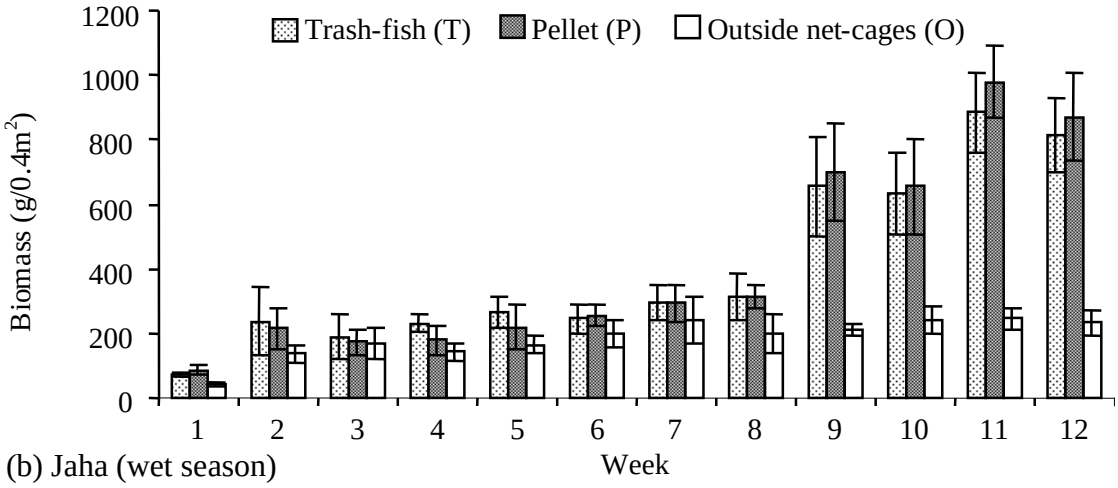
In Sangga Besar, mean wet biomass (g per 0.5 m^2 net panel dimension, g per panel) of sessile macrofoulers after eight weeks of development was significantly ($P < 0.001$) higher in pellet cages (535.33 g) compared to 344.36 g and 342.89 g in trash-fish and outside the net-cage respectively. The development rates were relatively higher in pellet cages than in trash- fish cages or outside the net-cage treatments (Figure 3.36c). Maximum wet biomass of 856.08 g obtained at the 8th week for pellet cages and 625.82 g on the 6th week in trash-fish cages. The maximum 519.03 g obtained at the 8th week outside the net-cages.

3.2.8.2. Non-sessile Associates

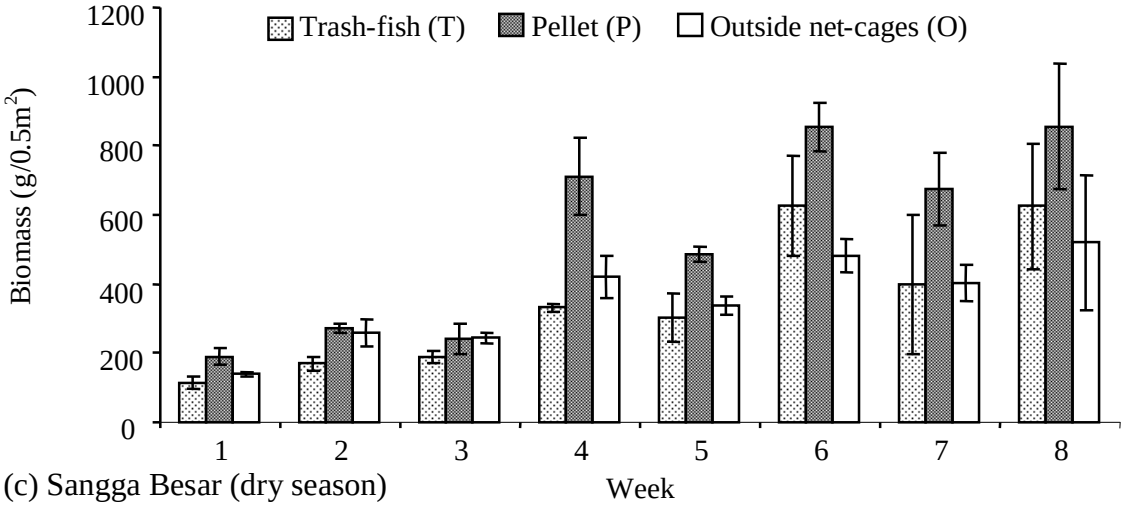
During the dry season at Jaha, mean wet biomass (g per 0.4 m^2 net panel dimension, or g per panel) of non-sessile macrofoulers after eight weeks of development was significantly ($P < 0.001$) higher in pellet (59.96 g) or trash-fish (56.14 g) cages compared to only 8.93 g outside the net-cages. A maximum biomass of 89.84 g and 84.11 g in pellet and trash-fish cages respectively was obtained at the 6th week. In contrast, maximum wet biomass outside the net-cages on 4th week was 14.13 g (Figure 3.37a) (Appendix 6b).



(a) Jaha (dry season)

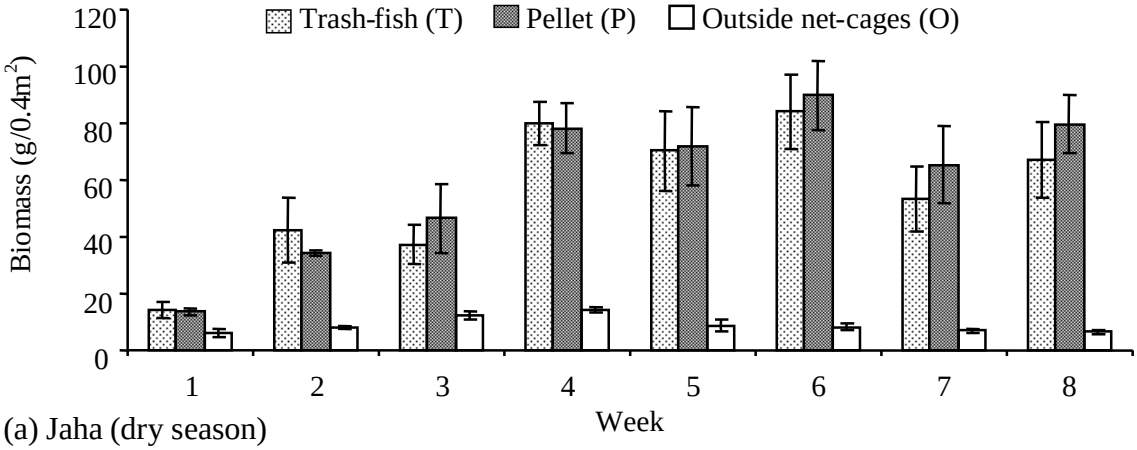


(b) Jaha (wet season)

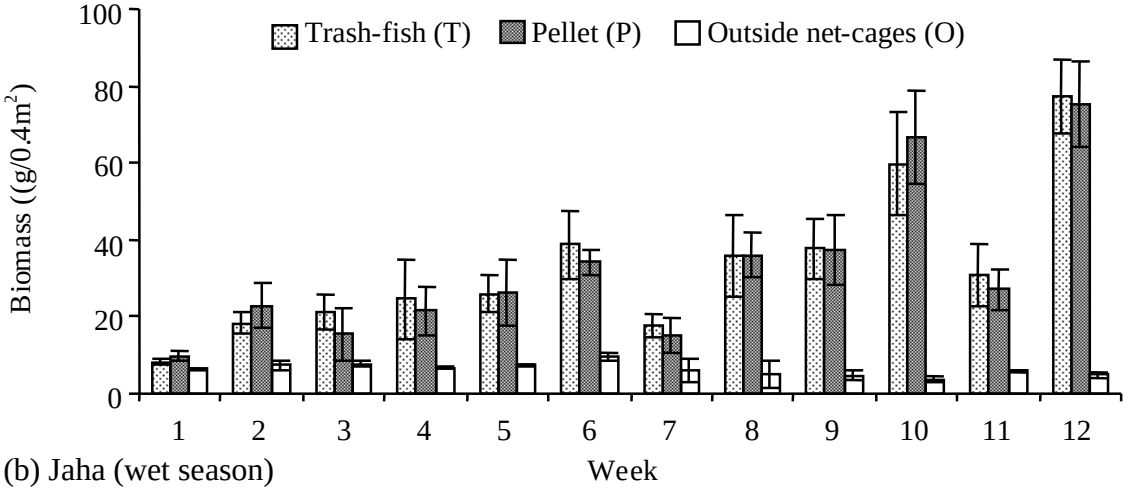


(c) Sangga Besar (dry season)

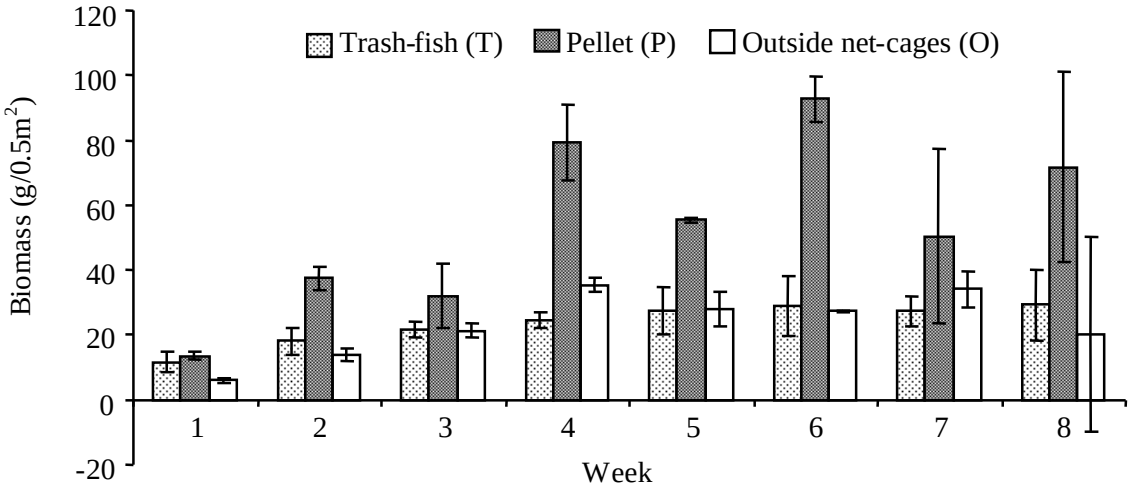
Figure 3.36. Weekly development in wet biomass (mean ± SD) of sessile biofouling organisms on net panels placed inside the net-cages given feed (pellet and trash-fish) and no feed outside the net-cages in a fish farm at (a) Jaha dry season, (b) Jaha (wet season) and (c) Sangga Besar (dry season).



(a) Jaha (dry season)



(b) Jaha (wet season)



(c) Sangga Besar (dry season)

Figure 3.37. Weekly development in wet biomass (mean ± SD) of non-sessile biofouling organisms on net panels placed inside the net-cages given feed (pellet and trash-fish) and no feed outside the net-cages in a fish farm at (a) Jaha (dry season), (b) Jaha (wet season and (c) Sangga Besar (dry season).

The eight-weeks comparison between the dry and wet seasons indicate that mean wet biomass (g per 0.4 m^2 net panel dimension) of non-sessile macrofoulers was significantly ($P < 0.001$) much lower during the wet season with 22.61 g, 24.17 g and 7.07 g respectively for pellet, trash-fish feed and outside the net-cages. Development rates were generally slower during the wet season than in the dry season. A maximum biomass of 75.19 g and 77.31 g respectively for pellet and trash-fish feed were obtained on 12^{th} week compared to less than 10 g outside the net-cages (Figure 3.37b).

In Sangga Besar, mean wet biomass (g per 0.5 m^2 net panel dimension) of non-sessile macrofoulers after eight weeks of development was significantly ($P < 0.001$) higher in pellet cages (54.04 g) than in trash-fish (23.59 g) or outside the net-cages (23.25 g). The increment rates were higher in pellet cages than in trash-fish or outside the net-cages. Maximum biomass of 92.67 g was obtained at 6^{th} week for pellet cages while it relatively constant at 28.05 g and 27.45 g for trash-fish cages and outside the net-cages respectively (Figure 3.37c).

3.3. DISCUSSION

3.3.1. Diversity of Macrofouling Assemblages on Nets

There were eight species of sessile macrofoulers including hydroids, seaweeds, barnacles and mussels on net panels immersed in floating net-cages at Jaha and Sangga Besar River estuary. The same sessile macrofouling organisms were found in Jaha and Sangga Besar farms, except that bryozoans were additionally encountered in Sangga Besar. The total number of non-sessile species ranged from 22 to 27 species depending on treatment feed, season and site, but the dominant species were species of amphipods, tanaidacea, nematodes and copepods. The number of macrofouling species is close to that recorded by Cheah & Chua (1979) who found 34 species including non-sessile organisms from one side of the net-cage (2.15 m x 1.45 m x 1.4 m) in the Penang Strait

after 2 – 4 months of immersion. However, their study included biofouling organisms on the wooden platform including the boring foulers such as *Martesia* spp.

In temperate waters, Braithwaite et al. (2007) recorded only 40 taxa of fouling organisms on salmon cage-nettings immersed over a 10-month period. Hodson et al. (2000) listed at least 18 species of biofouling organisms including non-sessile forms such as nereids, isopods and amphipods. Algae and ascidians dominated after 163 days immersion in salmon farms. Dubost et al. (1996) reported only 14 taxa of mainly plants and bryozoans in a freshwater aquaculture farm in Eastern France over a period of 28 – 69 days.

Most of the net biofouling species in fish farm at Jaha and Sangga Besar were common species that were frequently reported from other aquaculture systems of tropical, subtropical and temperate waters. Cheah & Chua (1979) recorded compound tunicates, mussels, oysters and algae as predominant forms in sessile macrofouling, but in this study *Plumularia* sp. and *Polysiphonia* sp. were the dominant species. There were no *Gracilaria* sp., *Bryopsis* sp., compound tunicates (Botryllus, *Botrylloides*, *Tridemnum*) and oysters (*Pinctada*, *Pteria*, *Crassostrea)* as recorded by Cheah & Chua (1979).

The barnacles, anthozoans, amphipods, polychaetes and isopods that were recorded by Cheah & Chua (1979) are similarly found in the present study, but *E. clathrata*, *Lyngbya* sp., *X. mangle*, tanaids, nematodes and copepods were not recorded in their biofouling list. Although Cheah & Chua (1979) did not mention the species composition of amphipods, they indicate that amphipods were among the highest in total abundance. *Gracilaria* was a common algae on the wooden structures of the fish farm in Sangga Besar but no species was encountered on the net panels during the study periods, possibly due to the smaller attachment surfaces of net panels and competition with the dominant species such as *Polysiphonia* sp., *Cryptosula* sp. and *Plumularia* sp..

Cnidarians (*Plumularia* sp. & unidentified anemones) and bryozoans (*Cryptosula* sp.) as recorded in this study are widespread and conspicuous macrofouling organisms on aquaculture structures. *Plumularia* sp. and *Cryptosula* sp. were the most dominant calcareous foulers of floating net-cages in Jaha and Sangga Besar. Several species of hydroids and bryozoans are important foulers of shellfish culture (e.g. Dharmaraj et al., 1987; Doroudi, 1996; Hughes, 2001; de Nys & Ison, 2004; Ross et al., 2004), salmon cages (Hodson et al., 2000) and other culture systems in tropical (Chua & Tech, 2002; Madin et al., 2009) as well as freshwater aquaculture (Dubost et al., 1996).

Relatively small population of barnacles and mussels were present on net panels. Barnacle is however a cosmopolitan biofouling organism, inhabiting temperate waters, subtropical and tropical waters of Southeast Asia including Malaysia (Zevina et al., 1992). The relatively low percentage cover of barnacles (*B. amphitrite*) and mussel (*X. mangle)* present in this study is probably due to the small attachment surface and intense competition with encrusting *Plumularia* sp. and anthozoans. Barnacles and mussels are important foulers of fish culture structure in tropical waters (Chua & Tech, 2002; Madin et al., 2009) and among the dominant species associated with shellfish farming in temperate regions (Minchin & Duggan, 1989; Ross et al., 2004).

Polysiphonia sp., *E. clathrata* and anthozoans were the important non-calcareous foulers of nets in Jaha and Sangga Besar. Cheah & Chua (1979) indicated that the biomass of algal fouling including *Polysiphonia* sp. was among the highest in their study. *Polysiphonia* sp. and *E. clathrata* are among the important foulers of salmon cage in Australia (Hodson et al., 1997; 2000), cultured oysters and other shellfish aquaculture in European waters (Enright et al., 1983). The fouling by algae is conspicuous in both fresh and marine waters, but marine algae are widely regarded as major worldwide fouling organisms (e.g. Fletcher, 1988; Callow, 1996; Finlay et al., 2002).

Several non-sessile macrofouling are also frequently listed in other biofouling studies in fish culture. Amphipods are among the important biofouling associates in shellfish culture as recorded by Ross et al. (2004) and in salmon cages (Hodson et al., 2000; Tan et al., (2002) in the temperate waters. According to Claereboudt et al. (1994), the biofouling associates of the sessile community on suspended culture of scallops include nematodes, errant polychaetes, amphipods, isopods, polychaetes and crabs. Almost all of these organisms were also found in the present study. However, tanaidacea and nematodes were rarely recorded, indicating that the tropical fish farm environment is more conducive for the development of non-sessile organisms.

Information on non-sessile macrofoulers on cage nettings and their effects are not well documented but their negative impacts may correlate with the sessile macrofouling community. For example tube dwellers and burrowers would give extra weight or stress to cage nettings. According to Ross et al. (2004) the silty tubes of amphipods proliferated on scallop cultivation nets after sea urchins had removed other biofoulers and it is thought to adversely affect the growth of scallops.

Organic matter invariably accumulates on net panels. Relatively higher accumulation of these materials may inhibit the development of sensitive species such as *X. mangle* due to higher burial rates, but on the other hand may have improved the growth and attachment strength of *Polysiphonia* sp. and *E. clathrata.* A number of biofouling studies on fish cages have indicated the high accumulation of unknown organic or mineral matter trapped on nets (e.g. Dubost et al., 1996; Hodson et al., 1997; Wu, 1995). Although their chemical composition is unknown, it is likely important to biofouling development and contributes to the fouled weight of net panels.

3.3.2. Seasonal Effects on Macrofouling Assemblages

The abundance of major macrofouling species and thus the total wet biomass of sessile

and non-sessile organisms respectively were significantly different between the dry and wet seasons in Jaha. The mean total wet biomass of both biofouling types inside the net-cages given feed (whether pellet or trash-fish) over eight weeks of colonization were significantly higher (at nearly two times) during the dry season than in wet season at Jaha. Their development rates were increased by a factor of 1.5 – 2 times from wet to dry season, with maximum biomass achieved by the 11^{th} and 6^{th} week respectively. The greater biomass and development rates of biofouling during the dry season is contributed mainly by the higher population of *Polysiphonia* sp., anthozoans, *E. clathrata*, *B. amphitrite*, amphipods and tanaids. Outside the net-cages, the biofouling population and total wet biomass were not significantly different between the dry and wet season because the net panels were dominated by similar macrofoulers of *Plumularia* sp. and *Gammaropsis* sp..

Several other studies indicate higher biofouling rates in fish cages as well as other artificial structures that coincided with the warmer season. According to Moring & Moring (1975) and Milne (1976), floating net-cage cultures are particularly vulnerable to biofouling during the hot season. Hodson & Burke (1994) and Hodson et al. (1995; 1997) indicate that salmon cages in Australia required net biofouling cleaning for every 5 – 8 days during summer due to higher biofouling level. Dubost et al. (1996) noted that the high level of biofouling during summer in freshwater aquaculture made it necessary to change the nets once a month but relatively low biofouling level in winter only required change once every three months.

The macrofouling species composition was generally not seasonally different between the dry and wet seasons, indicating that the year-round macrofouling of nets composed of similar species but the more favorable condition in the dry season significantly changed the species-specific abundance. Relatively minor seasonal alteration of physical and chemical parameters of tropical waters may or not change the

regular species composition, unlike in subtropical waters which experience distinct seasonal changes. Lin & Shao (2002) recorded a high diversity of biofouling during spring as compared to the fall season which corresponded to the main settlement season of several spring species reported by Soong et al. (1981).

Different seasons will show species-specific variations in abundance marine assemblages due to differences in larval recruitment, survival rate, growth rate and reproduction of the colonizers (e.g. Minchinton & Scheibling, 1991; Bertness et al., 1992; Underwood & Anderson, 1994; Saldanha et al., 2003). Furthermore, most species are known to produce larvae during a limited time and the season in which a substratum is submersed is expected to affect the colonization rates (Lin & Shao, 2002). Thus in the present study, seasonal difference in macrofouling abundance and total wet biomass could be due to different rates in reproduction of settled organisms and different availability, survival, recruitment and growth rates of the larvae, juveniles and spores of macrofouling during the dry and wet seasons. For example, higher population of barnacles and anthozoans during the dry season than in the wet season indicates higher availability of larvae in the estuarine waters that increases the chances of higher recruitment, settlement and growth rates on net panels.

Salinity and other parameters such as turbidity, temperature, pH, and oxygen were relatively different between the dry and wet season in Jaha estuary. Salinity is known to affect the stress level, osmoregulation, metabolism and growth rates of various marine organisms that will dictate their abundance (e.g. Perkins, 1974 cited in Bolduc & Aftons, 2003; Sousa, 1979; Barber & Blake, 1983; Barber & Davis, 1997; Grossman et al., 1998; Sagasti et al., 2001; Shriver et al., 2002). Biofouling organisms may respond differently to the various elements of water parameter changes during the dry and wet seasons that favor some species over others, depending on their sensitivity and tolerance. While non-sessile species may track preferred condition, sessile species must

either adapt or die.

Among the water parameters of Jaha, salinity was distinctively different between dry and wet seasons. The average surface salinity during the dry season was nearly 10 ppt higher than in the wet season, whereas surface turbidity was about 3 times higher. Salinity is well known to influence biofouling development with an optimal range favored for growth of particular species (e.g. Qiu et al., 1998; Witman & Dayton, 2001). Relatively high salinity during the dry season appeared to be more conducive for development of *Polysiphonia* sp., *E. clathrata*, anthozoans, *B. amphitrite*, amphipods and tanaids. For example, amphipods reproduce increasingly in higher salinities (Barnard & Gray, 1968 cited in Detwiler et al., 2002; Madin et al., 2009).

Furthermore, relatively higher salinity during dry season may have enhance the production of natural food and nutrients for marine biofouling species. Natural food such as phytoplankton grow more efficiently in higher salinity (Paerl et al., 2003), while organic carbon transport, cycling and uptake by marine organisms mostly occurred at higher salinity i.e. >25 ppt (Davies & Eyre, 2005). At the same period of the present study, Wong (2002) showed that mean chlorophyll-*a* levels were significantly higher ($P < 0.001$) during the dry season (45.8 µg 1^{-1}) than wet season (16.7 µg 1^{-1}). Thus marine planktons were more readily available in the water column for invertebrate macrofouling during the dry season.

In the wet season, surface and bottom salinities could differ by as much as 3 ppt over a depth of 3 m (see Table 3.1), but this difference may become more pronounced during episodic squally showers when surface salinity reaching 5 ppt had been recorded. Higher populations of *Plumularia* sp., *X. mangle*, nematodes and copepods during the wet season suggest their preference for lower salinity condition. On the other hand, their ability to quickly proliferate in varying salinities may give them the advantage to

flourish during the wet season. Many hydroids species have the ability to quickly proliferate in varying salinities (e.g. Folino, 2000; Slobdkin & Bossert, 2001; Bij de Vaate et al., 2002).

The developmental rates and abundance of fouling organisms decreases with decreasing salinity (e.g. Beveridge, 1987; Bombace et al., 1994: Laihonen et al., 1996), while most of the common fouling forms are unable to withstand low salinities that will impede the development of their larvae as well as the growth rate and maximum size attained (e.g. WHOI, 1952 cited in Yebra et al., 2003; Rosenberg, 1972; Cawthorne, 1978 cited in Kamer & Fong, 2000; Barber & Blake, 1983; Barber & Davis, 1997; Young, 1995; Gallager et al., 1996). This is also true from the present study, where low salinity stress during the wet season would reduce the population of biofouling organisms dominant during the dry season and thus reduce space competition resulting in propagation of the 'wet season' dominant species. For example, macroalgae were not as abundant during the wet season as was in the dry season despite the generally less turbid water suggesting that low salinity stress could reduce growth rates. Low salinity is known to stress algae in various aspects such as loss of pigmentation in *Enteromorpha* (e.g. Sfriso et al., 1987; Kamer & Fong, 2000). Furthermore, limitation of marine natural food and nutrients due to low salinity may have concomitantly reduced biofouling population and thus the biomass during the wet season (see also Mantoura & Woodward, 1983; Goni et al., 2003; Davies & Eyre, 2005).

Based on the findings of the present study, it is hypothesized that higher salinity (as observed during the dry season) is more suitable for marine biofouling forms thereby increasing the biofouling rates; conversely, low salinity (as observed during the wet season) decreases the biofouling rate and thus gives a lower biofouling biomass. However, further study is required to investigate the effects of salinity on the sessile macrofouling community structure in the tropical estuary (see Chapter 5, page 190).

3.3.3. Macrofouling Assemblages in Relation to Fish Rearing

Effect of Fish Rearing on Macrofouling Assemblages

With fish rearing and thus the input of fish feed, whether pellet or trash-fish, the abundance of major macrofouling species such as *Polysiphonia* sp., *B. amphitrite*, anthozoans, *E. clathrata*, amphipods and tanaids was significantly higher inside the net-cages than outside it. In both seasons at Jaha, total wet biomass of sessile and non-sessile biofouling organisms inside the net-cages given feed (pellet or trash-fish) was nearly three and six times higher respectively, as compared to outside the net-cages. In Sangga Besar, mean biomass of both biofouling types was significantly higher in pellet given net-cages (i.e. well maintained experimental net-cages) compared to outside the net-cages. These results suggest that fish rearing and thus the fish feed input in floating net-cages may significantly influence the development rates of biofouling assemblages on cages nets. However, further study is required to investigate the extent of the effect of fish feed and rearing on biofouling assemblages (see Chapter 4, page 166).

It has been reported that food wastage, organic and nutrient loadings were several folds higher when minced trash-fish was used instead of pellet feed (e.g. Warren-Hansen, 1982a; Ove-Arup, 1989; Hansen et al., 1990; Wu, 1995; Qian et al., 2001). Higher productions of food wastage, organic and nutrient loadings in trash-fish feed are expected to attract higher biofouling level. However, the pellet and trash-fish feed generally had similar species composition, abundance and total wet biomass of sessile macrofouling and non-sessile organisms respectively. Higher biofouling in net-cages given pellet feed are possibly due to inferior formulation including poor binding properties and water stability. The home-made pellets contained a high percentage of unbound fine powder and easily break up during transportation and feeding. Their release into the water column inside the net-cages, probably encouraged the growth of suspension feeders such as barnacles, mussels and deposit feeders like amphipods,

tanaids and nematodes. It is thus hypothesized that the use of higher quality pellet feed including better water stability and vigorous texture could reduce fish feed wastage and thus the development of biofouling assemblages. Further study is required to investigate the effects of different quality of pelleted feed on net macrofouling development (see Chapter 4, page 166).

Organic enrichment from fish culture activities including uneaten fish feed particulate and other waste material may increase the nutrient levels and therefore the biofouling assemblages inside the net-cages. In the same farm in Jaha River estuary, Alongi et al. (2003) showed higher concentrations of NH_4^+, PO_4^3, and $NO_2^- + NO_3^-$ inside the net-cages than in non-cages sites. The higher concentrations of nutrients may enhance higher rates of algal fouling such as *Polysiphonia* sp. and *E. clathrata*. According to Folke et al. (1994), the dissolved organic fraction from aquaculture is directly available to algae. For example, *Enteromorpha* can show undesirable growth in respond to nutrient enrichment (Burkholder et al., 1992). Nonetheless, nutrients enrichment or eutrophication had been suggested as an important factor that influenced biofouling development (e.g. Meyer-Reil & Koster, 2000; Mayer-Pinto & Junqueira, 2003).

The direct effect of organic or nutrient enrichment on sessile epifauna is however not well understood, although it is well known to enhance phytoplankton blooms which in turn provide food for invertebrate larvae and enhance the growth rate of settled suspension feeders such as *Plumularia* sp., barnacles, anthozoans and mussels. Further study on nutrient and chlorophyll-*a* concentrations of culture water inside the net-cages is however required so as to determine their possible effects on the biofouling assemblages (see Chapter 6, page 206).

Fish rearing in floating net-cages could generate foods that elicit a wide range of

feeding strategy among the non-sessile macrofouling organims. The availability of food can affect reproduction, recruitment rate and stimulate the migration of marine animals toward the areas where resources of food are more abundant (Hughes, 1993). Amphipods for example consume a wide range of estuarine particles by filtering and deposit feeding on sediment and detritus (Dewitt et al., 1992) or scavenging on animal foods (Britton & Morton, 1994; Cruz-Riveira & Hay, 2000a; 2000b). These could well be practiced by the dominant *Gammaropsis* sp. and *Photis* sp. on uneaten fish feed particulates and other waste products (Madin et al.,. 2009). Coprophagy commonly observed in copepods, e.g. *Oithona* spp. (Gonzalez & Smetacek, 1994), could well be practiced by macrofouling fauna feeding on the faeces of cultured fish.

Other Concomitant Effects of Fish Rearing on Macrofouling Assemblages

The net panels suspended inside the net-cages given pellet or trash-fish (Plate 3.3) feed were covered almost completely by organic matter of not obvious origin within the first four weeks of immersion, resulting in a more heterogeneous surface that may attract settlement of biofouling organisms as compared to net panels hung outside (Plate 3.4) the net-cage. According to Connell (2001), structural complexity and composition of the substratum will influence the macrofouling communities. A more complex and heterogeneous structure offers a greater array of niches that provide a hiding place to avoid predation or cannibalism, thus will allow less competitive organisms to recruit as well as increases the hatching success of larvae and juvenile (e.g. Menge, 1976; Bailey & Houde, 1989; Rilov & Benayahu, 1998; Duedall & Champ, 1991; Svane & Petersen, 2001; Holbrook et al., 2002).

The higher population and thus the biomass of macrofouling assemblages inside the net-cages than outside it could also be due to low rates of grazing and predation as compared to net panels placed outside the net-cages. Several species of wild fish

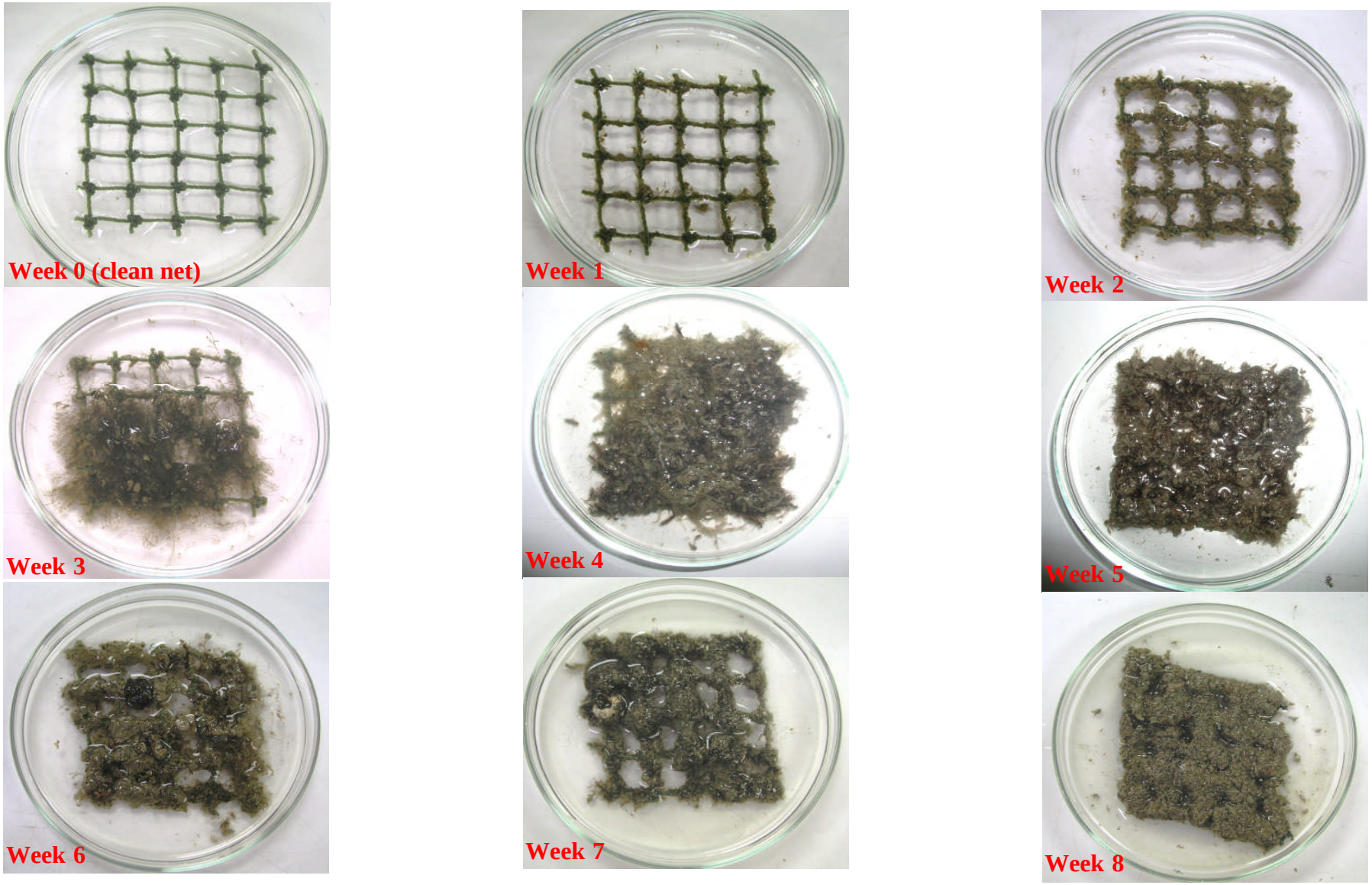


Plate 3.3. Weekly (week 0 – 8) progression of macrofouling assemblages on net panels placed inside the net-cages as shown from the sub-panels (5 x 5 cm). Net filaments were almost completely covered by organic matter of undetermined origin present amongst the macrofouling organisms within the first four weeks of immersion (e.g. dry season).

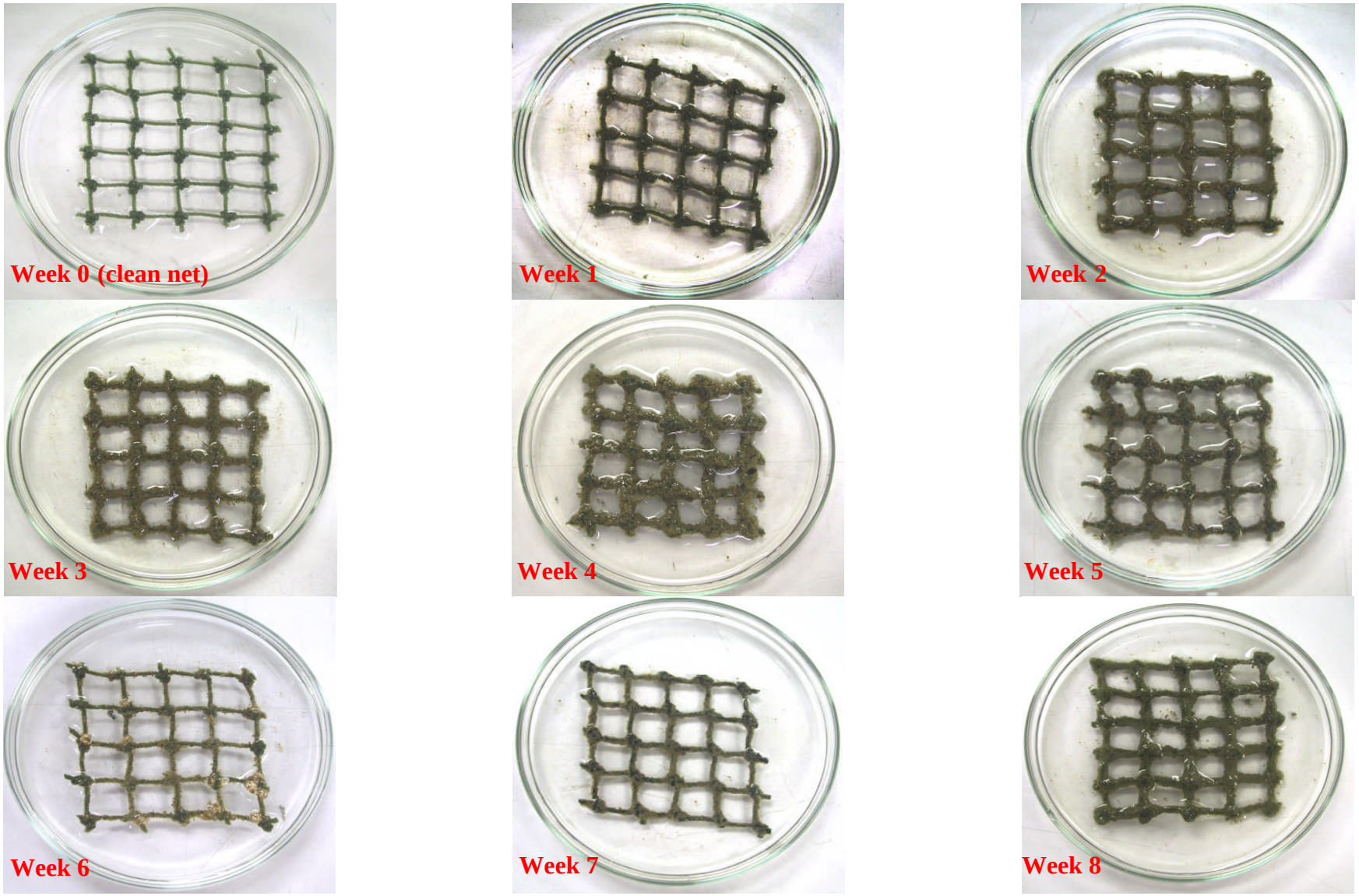


Plate 3.4. Weekly (week 0 – 8) progression of macrofouling assemblages on net panels placed outside the net-cages as shown from the sub-panels (5 x 5 cm). Net filaments covered by relatively small amounts of organic matter present amongst the macrofouling organisms comprising mainly of *Plumularia* sp. (e.g. dry season).

including predators and grazers such as arrid fish and scatophagid fishes are commonly found in a fish farm vicinity of MMFR. Other major predators of biofouling organisms such as barnacles, anemones, mussels and bryozoans include fish and crabs (Rheinhardt & Mann, 1990), flatworms (Branscomb, 1976), snails and nudibranchs (Marsh, 1976). Nevertheless, grazing or predation is well known as an important factor in structuring biofouling assemblages in aquaculture (e.g. Greene & Grizzle, 2007).

The relatively low population of several sessile and non-sessile biofouling species outside the net-cages could also be due to stress from strong water flow and lower amount of food. Water flow rate is known to affect biofouling in many ways. According to Nikora et al. (2002a; 2002b; 2002c), the flux of phytoplankton for suspension filter feeders and the supply of nutrients to aquatic plants are influenced by the interaction of the organisms and the water flow. In the present study, strong water flow (>20 cm s^{-1}) outside the net-cages may reduce the food availability such as phytoplankton and result in lower biofouling rate and biomass. There is reduced chance that biofouling organisms could catch and assimilate their potential food in strong water flow. In addition, strong water flow also detaches settled larvae and spores and possibly disrupts the colonies and habitats of non-sessile biofouling organisms such as tube dwellers. In contrast, relatively slow water flow rates (<10 cm s^{-1}) inside the net-cages increase the residence time of biofouling larvae and give opportunity for their attachment on net panels. Study on the water flow dynamics in floating net-cages is however required to determine its specific effect on the biofouling assemblages (see Chapter 4, page 166).

3.3.4. Depth Distribution of Sessile Macrofouling Organisms

Sessile macrofouling on net panels is generally not depth dependent but intense competition may have caused species to concentrate at a particular depth stratum. Generally, the number of species and percentage cover occupied by the sessile

community were higher at the uppermost (≤ 0.83 m) and middle (1.0 – 1.66 m) strata as compared to the bottom stratum (1.66 – 2.5 m) of the net panels in Jaha and Sangga Besar. However, competition for the limited space and other resources resulted in a smaller percentage cover of each individual species. Several other studies indicate higher biofouling at the near surface water, for example Moring & Moring (1975) observed higher biofouling at the near surface (50 cm) of salmon cages in temperate waters. The highest population of biofouling organisms in freshwater aquaculture occurred at the 0.4 – 1.0 m depth (Dubost et al., 1996).

The depth distribution of macrofouling organisms appears to be seasonally structured and influenced by fish rearing activities. During the dry season at Jaha, *Polysiphonia* sp. and *E. clathrata* were prominent at the top stratum which indicates their requirement of light for photosynthesis. Hodson et al. (1997) similarly found a higher population of several algal foulers including *Polysiphonia* sp. at the shallower depth (1 – 1.5 m) of salmon cages in Australia. According to Kangas et al. (1982) and Cloern (2001), low light availability will limit macroalgae population by decreasing their downward depth distribution. This suggests that low light level at the middle (0.66 m depth) and bottom (2 m depth) of the net-cage may prevent active growth of algal species and thus allow the intense colonization and proliferation of invertebrate such as anthozoans, *B. amphitrite* and *Plumularia* sp.. Light attenuation at or near the net-cage bottom is possibly due to high amount of suspended material thereby increasing turbidity.

In the wet season at Jaha, low salinity stress experienced particularly by *B. amphitrite*, anthozoans, *E. clathrata*, *Lyngbya* sp. and *Polysiphonia* sp. had resulted in the domination of *Plumularia* sp. at the middle and near bottom stratum of net panels inside the net-cages. Although the *X. mangle* could proliferate in low salinity condition, its cover was relatively reduced with increasing net-cage depth probably due to

smothering from high accumulation of organic wastes which would reduce the survival of its larvae at the cage bottom. The concentration/thickness of organic matter was relatively higher at the bottom stratum than at the surface and middle strata of net panels placed inside the net-cages.

Outside the net-cages, the net panels were almost completely dominated by the *Plumularia* sp., in the dry season and more so during the wet season, from top to bottom (0 – 2 m depth) in Jaha. This suggests that its small form, encrusting mode of growth and ability to quickly proliferate in varying salinities confer it a competitive edge living under strong water flow and lower salinity condition during the wet season. Small *Lyngbya* sp. proliferated outside the net-cage during the dry season, further indicating that smaller form organisms encounter less shear stress from strong water flow.

In Sangga Besar, the upper stratum of net panels was dominated by *Plumularia* sp. but other species such as *Polysiphonia* sp., *E. clathrata* and *X. mangle* were also present with smaller percentage cover possibly due to intense competition for limited attachment surface. Unlike that in Jaha, anthozoans and *Plumularia* sp. along with the vigorously growing *Cryptosula* sp. were competing among each other for the limited attachment surface of the middle and bottom strata of net panels in Sangga Besar. Outside the net-cages where strong water flow conditions prevailed, the net panels were almost completely dominated by small forms such as *Plumularia* sp. and *Cryptosula* sp., from top to bottom (0 – 2.5 m depth).

The orientation of the net panel may also influence biofouling, in that the top part of the net panels in Jaha (upper 1.33 m) and in Sangga Besar (upper 1.66 m) of net panels was vertically positioned similar to the sides of the net-cage, while the bottom part horizontally resting on to the net-cage bottom, assumed a similar orientation as the cage bottom. The macrofouling population was generally higher on the upper vertical part of

net panels as compared to the bottom or horizontal part. The higher sedimentation on the horizontal part of the net panel may have discouraged the growth of certain biofouling species, while more tolerant species such as *Plumularia* sp. was not affected (see also Baynes & Szmant, 1989; Cortes & Risk, 1985; Brodie, 1997; Wesseling et al., 1999; Wendt et al., 1989; Clark & Edwards, 1999).

Unsuitable water conditions at the net-cage bottom could have reduced the number and percentage cover of biofouling species dominated by *Plumularia* sp.. Dissolved oxygen and pH value were relatively lower at the net-cage bottom which had higher turbidity. There were no indication of extreme dissolved oxygen depletion or hypoxia but concentration was likely lower at night. Longer periods of hypoxia can cause mass mortality of sessile organisms and could change species composition (e.g. Jorgensen, 1980; Stachowitsch, 1984; Josefson & Widbom, 1988; Llanso, 1992). These suggest that the ability of *Plumularia* sp. to colonize the bottom stratum may indicate its tolerance to low dissolved oxygen concentration.

3.3.5. Weekly Colonization of Macrofouling Organisms

In Jaha, the colonization rates of most macrofouling species were relatively slower during the wet season than in the dry season but this was much dependent on species rather than the entire population. The sessile macrofouling community began with encrusting *Plumularia* sp. while the non-sessile macrofouling community began with *Gammaropsis* sp.. Various authors have reported that that the earliest colonizers of artificial structures have a selective life history strategy such as high fecundity, rapid growth, early sexual maturity, resistance to predation, ability to reproduce repeatedly and disperse large numbers of larvae or juveniles (e.g. Borowsky, 1980; Hughes, 1983; Fredette & Diaz, 1986; Turner & Todd, 1993; Butler & Connolly, 1996; Barnes & Hughes, 1999; Block et al., 2003; Hobson & Chess, 1976 cited in Taylor, 1998;

Hammer & Zimmerman, 1979 cited in Taylor, 1998; Takeuchi & Hirano, 1992a; 1992b). In the present study, the population of *Plumularia* sp. and *Gammaropsis* sp. could reach near maximum numbers within the first four weeks of net panel immersion suggesting that both species have the life strategy as above.

Colonization and community development of sessile macrofouling organism was seasonally modified as well as influenced by the fish rearing activities. During the dry season at Jaha, macrofouling species such as *Polysiphonia* sp., anthozoans and *B. amphitrite* only started to develop after the initial colonization by *Plumularia* sp. but their progressive development gradually surpassed that of *Plumularia* sp. for both treatments inside net-cages given feed (Figure 3.38). Higher development rate of *Polysiphonia* sp. at the upper stratum of net panels caused gradual decrease of *Plumularia* sp., while higher development rates of anthozoans and *B. amphitrite* at the middle and bottom strata reduced the number of *Plumularia* sp. These indicates that relatively tiny forms of encrusting species such as *Plumularia* sp. are easily overgrown by the bigger form and aggressive colonizers such as *Polysiphonia* sp., anthozoans and *B. amphitrite.*

However during the wet season at Jaha, domination by *Plumularia sp*. was more pronounced and it dominated throughout the study period (Figure 3.39). Its competition with *Polysiphonia* sp. and *X. mangle* for space appeared intense at the upper strata but there was relatively no competition at the middle or bottom strata of the net panels inside the net-cages. Although *Polysiphonia* sp. tolerated the low salinity during the wet season, its cover at the surface decreased when *X. mangle* proliferated near the end of experiment (i.e. week 6^{th} onwards) suggesting that the latter is more resistant to the lower salinity condition. The vigorous growth of *Plumularia* sp. during the wet season subsequently overwhelmed the low-salinity stressed species such as anthozoans and *B. amphitrite* (see also Madin et al., 2009).

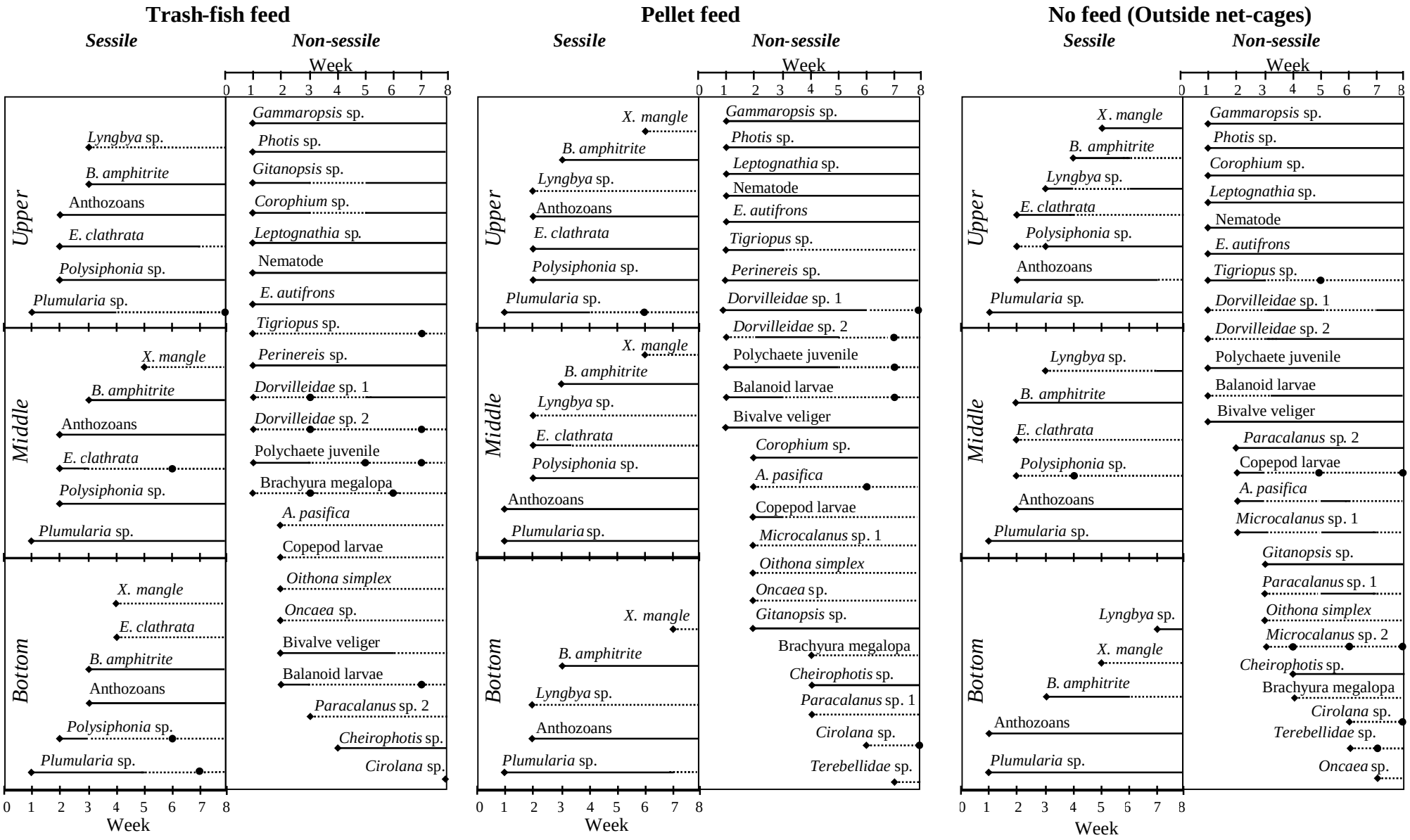


Figure 3.38. Schematic diagram showing colonization sequence of macrofouling organisms on net panels placed inside the net-cages given trash-fish feed, pellet feed and no feed (outside the net-cages) during the dry season in a fish farm at Jaha. Continuous solid line indicate a continuous presence of species, symbol '•' indicate species only present at a point (i.e. week) while a dash line indicates absence of individuals.

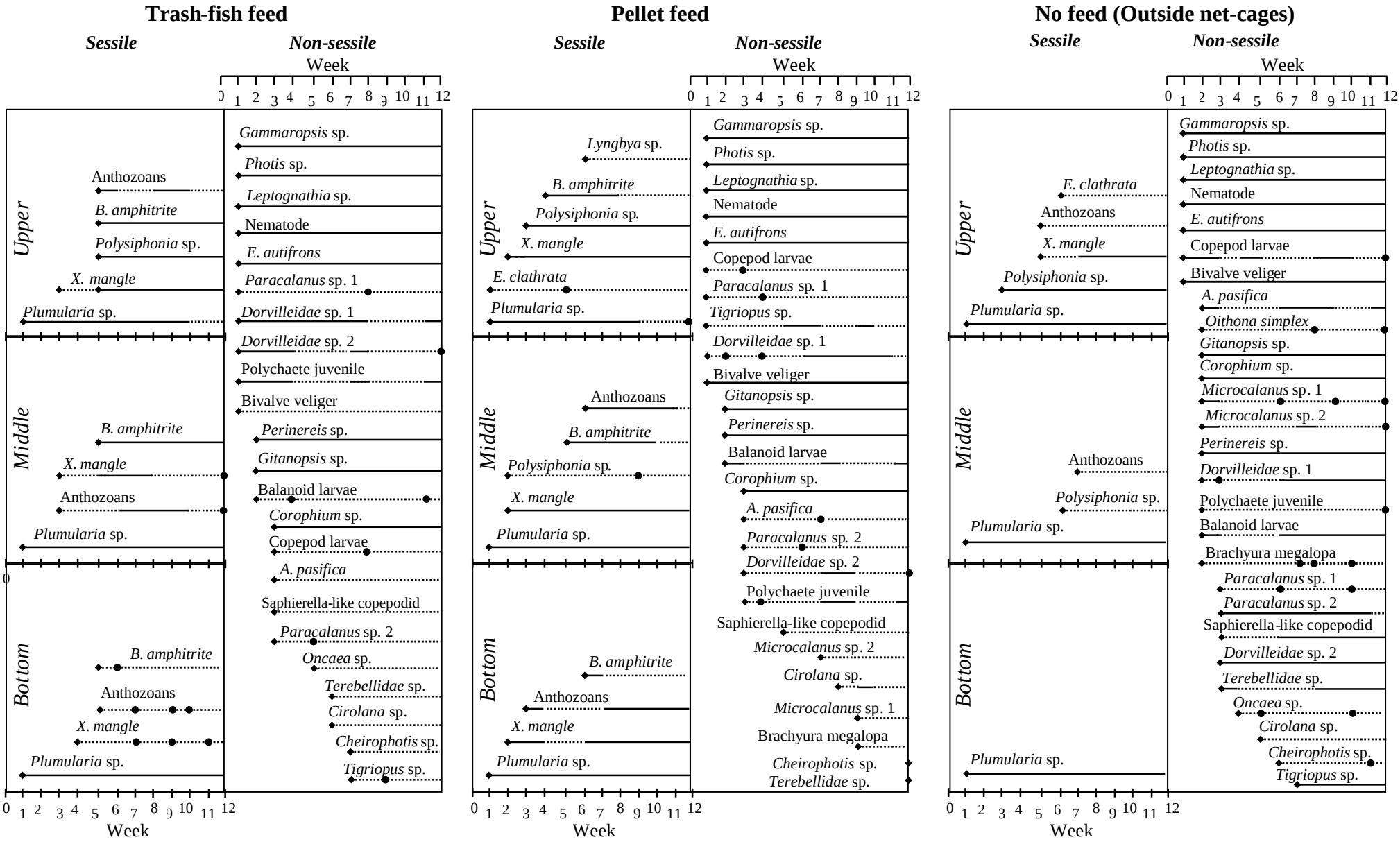


Figure 3.39. Schematic diagram showing colonization sequence of macrofouling organisms on net panels placed inside the net-cages given trash-fish feed, pellet feed and no feed (outside net-cages) during the wet season in a fish farm at Jaha. Continuous solid line indicate a continuous presence of species, symbol '•' indicate species only presence at a point (i.e. week) while a dash line indicates absence of individuals.

Outside the net-cages, space competition among the sessile macrofouling organism were relatively low. In both seasons at Jaha, low development rates by other sessile macrofouling organisms resulted in the domination by *Plumularia* sp. throughout the study particularly at the middle and bottom strata. This further suggests the considerable affect of strong water flow, predation and limited amount of food on macrofouling assemblages outside the net-cages (see previous).

For both seasons in Jaha, the abundance of *Gammaropsis* sp. inside the net-cages was gradually reduced as Photis sp. progressively increased in numbers. However, outside the net-cages, *Gammaropsis* sp. appeared to be the dominant colonizers without competition from *Photis* sp.. This suggests that *Photis* sp. was attracted to net-cages due to additional organic inputs from uneaten food and fish faeces, as well as the much reduced water flow inside the net-cages (see also Madin et al., 2009). In addition, the relatively smaller physical size of *Photis* sp. as compared to *Gammaropsis* sp., could make it more adaptable as a tube dweller on the net panels. Colonization rates of tanaids, nematodes, amphipods species were relatively slower and often surpassed by the dominant *Gammaropsis* sp. or *Photis* sp..

Although the competition between *Gammaropsis* sp. and *Photis* sp. did not occur outside the net-cages, abundance of *Gammaropsis* sp. was reduced near the end of the experiment (5 – 6th week) suggesting that they had reached their peak. Other factors such as cannibalism or predation may also be responsible for the decrease in population. Cannibalism and intraguild predation in amphipods have been noted by Otto (1998) and MacNeil & Prenter (2000). Most of the amphipods encountered were juveniles which were vulnerable to cannibalism.

In Sangga Besar, *E. clathrata* and *Plumularia* sp. were the early colonizers within first week of immersion during the dry season (Figure 3.40). In all treatments,

Plumularia sp. seem to dominate throughout the study but intense competition with *E. clathrata* and *Polysiphonia* sp. at the upper and middle strata gradually reduced its population. Competition with anthozoans and *Cryptosula* sp. at the middle and bottom strata had gradually reduced *Plumularia* sp. near the end of the experiment. There were no clear competition between *Gammaropsis* sp. and *Phostis* sp. in Sangga Besar whether inside or outside the net-cages, possibly due to the relatively low abundance of *Gammaropsis* sp. and low amount of food available after a portion of the stocked fish died.

The final community of sessile macrofouling was influenced by season and fish rearing activities. During the dry season at Jaha, the sessile macrofouling community inside the net-cages had a final species for a particular depth. *Polysiphonia* sp. was the main final species at the upper stratum after out competing *Plumularia* sp.. An unidentified anemone was the final species at the middle and bottom strata. During the wet season, *Polysiphonia* sp. or *X. mangle* was the main species at the upper stratum but there was relatively less competition for *Plumularia* sp. which became the main species at the middle and bottom strata of net panels placed in feed given net-cages. Outside the net-cages, *Plumularia* sp. and *Gammaropsis* sp. appeared to be the two initial colonizers and final main species of the macrofouling community regardless of season and depth strata. Both species are dominant throughout the study period.

Net panel colonization by non-sessile organisms appears to be related to the development of the sessile organisms. Their populations and thus the total wet biomass always increased in tandem with each other (see Figure 3.4), suggesting that sessile macrofoulers besides increasing the surface area for non-sessile species also entrap food particles and may provide shelter against predators. On the other hand, reworking of organic waste by non-sessile organisms could provides higher nutrient for sessile macrofouling organisms (see also Madin et al., 2009).

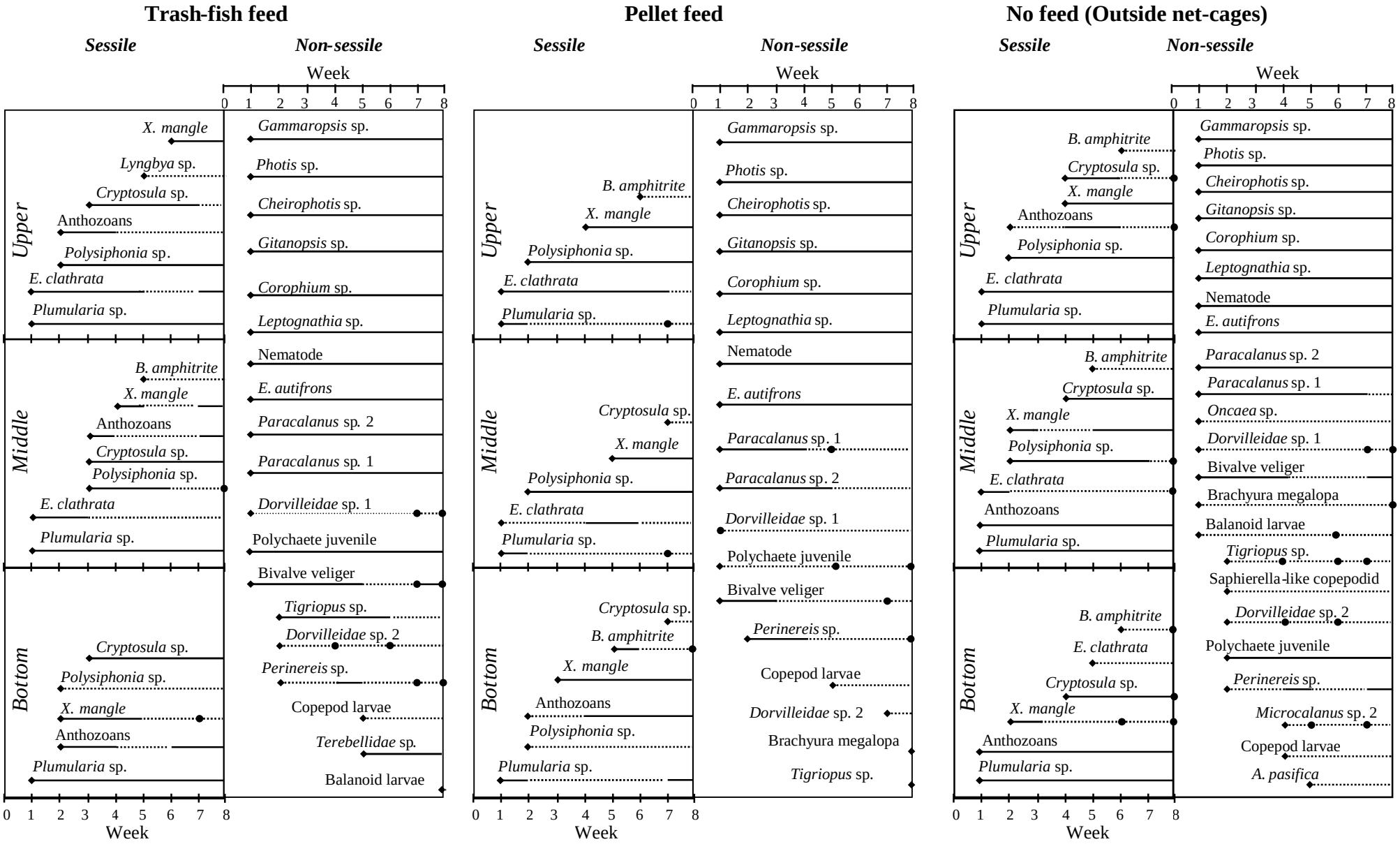


Figure 3.40. Schematic diagram showing colonization sequence of macrofouling organisms on net panels placed inside the net-cages given trash-fish feed, pellet feed and no feed (outside net-cages) during the dry season in a fish farm at Sangga Besar. Continuous solid line indicate a continuous presence of species, symbol '•' indicate species only presence at a point (i.e. week) while a dash line indicates absence of individuals.

3.3.6. Effects of Other Factors

Mean percentage cover of bryozoan and algal fouling was relatively higher in Sangga Besar than in Jaha. Chong et al. (1999) reported that Sangga Besar estuary was slightly polluted by various factory and agricultural activities located upstream. The more polluted environment may enhance the biofouling development in floating net-cages (see also Madin & Chong, 2004). According to Mayer-Pinto & Junqueira (2003), organic pollution will change the relative dominance of species in the fouling communities. Furthermore, current velocity at the fish farm was significantly reduced in Sangga Besar due to obstruction by dense net-cages. These factors may contribute to the much higher percentage cover of sessile macrofoulers (i.e. *Cryptosula* sp.). Modifications on water movement will change the sessile marine community and abundance (Turner et al., 1997).

Higher abundance of *Plumularia* sp. in Jaha may be due to its shallow water and relatively low salinity than in Sangga Besar. According to Gallager et al. (1996), Madin et al. (1996) and Concelman et al. (2001), shallow and well mixed water will enhance hydroid population. More macrofouling larvae are likely to reach and settle on the net panels at Jaha estuary with it low fish farm density and proximity to the river mouth.

Total abundance of non-sessile macrofouling organism on net panels placed outside the net-cages was higher in Jaha than in Sangga Besar. This suggests that the more optimal water parameter condition such as salinity at Jaha may be more conducive for their development. Furthermore, the use of antibiotics and pesticides to treat fish parasites and other diseases in aquaculture farms could have reduced non-sessile and other invertebrates that are responsible for macrofouling. Large-scale net-cage operation in Sangga Besar will use a higher amount of pesticide than in Jaha. Pesticides have been shown to affect reproduction and development of marine invertebrates (Levin et al.,

1996; Lee & Oshima, 1998; Hansen et al., 1999) particular on molting cycles of target species (Strawbridge et al., 1992; Green et al., 1996; Sanger et al., 1999).

The estimation of natural (without net-cage) net biofouling rate was 176 g m^{-2} wk^{-1}, but with fish rearing (with feed input) could increase the net fouling rate to nearly 350 g m^{-2} wk^{-1} during the dry season while in the wet season, the rates are estimated at 55 g m^{-2} wk^{-1} and 128 g m^{-2} wk^{-1} for natural (without cage) and inside the net-cages biofouling, respectively (Table 3.11). These rates are relatively higher than the net fouling rates of 93.3 g m^{-2} wk^{-1} in Penang Strait (29.9 ppt salinity) which estimated from the results of Cheah & Chua (1979). These authors did not mention the area of net sampled but they cleared all sessile organisms from one side of a 2-month old cage (215 x 145 x 140 cm) which weighed 2,238.7 g.

Table 3.11. Summarized data of highest mean biomass of sessile and non-sessile biofouling organisms by season (wet and dry) and treatment (outside net-cage O, pellet P and trash-fish T) in a fish farm at Jaha. Submersion period in weeks is given in parentheses. Mean biofouling rate based on total weight (g) on 1 m^2 of net panel per week. Area of experimental net panel used = 0.4 m^2.

	Biofouling rates (g per m^2)			Mean rate ($g\ m^{-2}\ wk^{-1}$)
	Sessile	Non-sessile	Total	
Dry season				
Outside net-cage, no fish and feed, O (4)	670	35	705	176
Fish given pellet feed, P (6)	2,095	227.5	2,322.5	387
Fish given trash-fish feed, T (5)	1,705	175	1,880	376
Wet season				
Outside net-cage, no fish and feed, O (11)	610	15	625	58
Fish given pellet feed, P (11)	2,465	67.5	2,532.5	230
Fish given trash-fish feed, T (12)	1,535	192.5	1,727.5	143

3.4. CONCLUSION

Macrofouling assemblages of cage nettings including their species abundance, colonization dynamics, depth distribution of sessile organisms, total wet biomass and

development rates were influenced by fish rearing and season. With fish rearing and thus the fish feed input, macrofouling populations inside net-cages generally increased, while outside the net-cages, particular species such as *Plumularia* sp. and *Gammaropsis* sp. dominated. With higher salinity as in the dry season, the populations of *Polysiphonia* sp., *E. clathrata*, anthozoans (unidentified anemone), *B. amphitrite*, amphipod and tanaids increased. Low salinity in the wet season gave higher populations of *Plumularia* sp., *X. mangle*, nematode and copepods. Due to competition and available larval pools, the macrofouling species exhibit vertical distribution and succession which are modulated by the effects of fish culture. Further studies on the effects of fish rearing (including feed input), water flow dynamics, salinity and nutrient concentrations, on the rate of biofouling are necessary to better understand the biofouling problem on net-cage in order that it could be controlled.

CHAPTER 4

EFFECT OF FISH REARING, FISH FEED, WATER FLOW AND NET-CAGE POSITION ON FOULING BIOMASS

Summary of Important Finding

The proposed hypothesis that fish feed with high water stability and less fine particulate content contributes to lower biofouling on cage nettings than fish feed of low water stability and high particulate content was not accepted. Use of high quality pellet feed (i.e. commercially-produced extruded pellet, treatment M) vis-à-vis low quality fish feed (i.e. steamed home-made pellet feed, treatment P and trash-fish feed, treatment T) did not confer reduction of both sessile and non-sessile biofouling organisms. A reduced flow rates to less than 10 cm s^{-1} inside the net-cage will significantly encourage the rapid development of sessile biofouling biomass (g m^{-2} wk^{-1}), with (175 – 231% higher in treatments M, P & T) or without (56 – 145% higher in treatments N) fish rearing and feed input compared to swifter water flow i.e. >25 cm s^{-1} outside net-cages (C) after 8 wk of development. However, non-sessile organisms were more attracted to the organic inputs from fish rearing inside the net-cages (i.e. whether M, P & T); their biomass (g m^{-2} wk^{-1}) were 459 – 802% higher compared to treatments without fish rearing and feed input (N) or outside net-cages (C) after 8 wk of development. This indicates that organic enrichment from fish rearing enhances the development rates of non-sessile organisms on nets. Biofouling on cage nettings significantly reduced water flow inside the net-cages. However, the net-cage units themselves (without biofouling) also play a significant role in flow attenuation. There were no significant ($P > 0.05$) effects on biofouling due to net-cage position, both longitudinally and transversely, suggesting that the reduced water flow by and in the net-cages is rather consistent throughout the farm.

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4.1. INTRODUCTION

Based on the results reported in Chapter 3, abundance and total wet biomass of biofouling assemblages were significantly higher on net panels placed inside the cages given fish feed (pellet & trash fish) than on net panels placed outside the net-cages (no fish feed input). The result suggests that fish rearing along with fish feed input and retarded water flow inside the net-cages significantly enhanced macrofouling. However, the extent of their effects on macrofouling was not assessed. Therefore, the purpose of this study was to determine the effects of fish rearing, fish feed input and water flow on the development of biofouling assemblages. Results from Chapter 3 also lead to the hypothesis that the use of high quality pellet feed including better water stability, vigorous texture and optimal nutrition reduce fish feed wastage and thus the development rates of biofouling assemblages. Thus, an important purpose of this chapter was to test the proposed hypothesis.

There are various reasons thought to influence biofouling development on fish cages, however several authors indicate that waters of fish farms are rather conducive to rapid fouling development due to increased level of nutrient and organic loadings (e.g. Ruokolahti, 1988; Dubost et al., 1996; Hodson et al., 1997; Coasta-Pierce & Bridger, 2002; Cook et al., 2006). Fish feed and feeding is an important source of nutrient and organic loadings of caged fish culture. Cage culture itself is always characterized by the higher proportion of feed loss and higher amount of effluents attributed to feed wastage (e.g. Cho et al., 1994; Wu, 1995; Coasta-Pierce & Bridger, 2002; Islam, 2005).

The published estimates of feed wastage from caged fish culture operation are however limited owing to the difficulties in separating the uneaten food from the other solid wastes (e.g. Merican & Phillips, 1985; Beveridge et al., 1991; Seymour & Bergheim, 1991; Handy & Poxton, 1993). The amount of feed that goes uneaten and

sinks as a waste is estimated to vary between 1% and 40%, but depends on the type of feed, feed quality, original composition of feed, feeding method, stocking density and feeding rate (Thorpe et al., 1990; Beveridge et al., 1991; Findlay & Watling, 1994).

The use of low quality feed is known to produce higher amount of wastage including uneaten feed and other solid particles. A number of authors indicate that food wastage were several folds higher when minced trash fish feed are used instead of commercial pellet feeds (e.g. Warren-Hansen, 1982; Ove Arup, 1989; Hansen et al., 1990; Wu, 1995; Qian et al., 2001). APEC/NACA/BOBP/GOI (2002) reported that solid wastes produced from the use of trash fish feed was 40% higher than the use of pellet feed. High amount of feed wastage could result in a huge loading of nutrient and organic matter (Karakassis et al., 2000; Carroll et al., 2003). The estimated value of nutrient loading from trash fish wastage is as high as 320.6 kg/ton compared to the range of between 47.3 and 130 kg/ton for commercial diet (Islam, 2005). This indicates that the higher amount of feed wastage from both trash fish feed and the inferiorly formulatated home-made pellet feed could have enhanced biofouling development as reported in Chapter 3.

The actual amount of supplied feed that is consumed by the fish and its digestibility are among the important factors influencing the waste output from cage culture operation. Butz & Vens-Cappell (1982) calculated a faecal production of 260 g dry weight of faeces per kg of feed consumed and 26% of the food eaten that ends up as faeces. With poor feed quality such as trash fish feed, the amount of feed eaten that ends up as faeces will be increased (Ackefors & Enell, 1994; Cho et al., 1994; Nijhof, 1994; Talbot & Hole, 1994), due to poor food conversion ratio (Leung et al., 1996).

Islam (2005) highlighted that feed wastage and hence the nutrient loads from mariculture in Asia were much higher due to the predominant use of low quality feed

such as trash fish diet unlike the temperate regions where high quality pellet feed are commonly used. This suggests that biofouling is more prominent in Asia due to the use of improperly processed trash fish diet and other low quality home-made fish feed products. However, trash fish feed is still widely used in Asia due to poor understanding of the nutritional requirements of species cultured (Wu, 1995) and resource-poor farmers (ADB/NACA, 1998). Among other reasons is the lower price and well established supply as it is readily available from local fishermen by catch (Che Musa & Nuruddin, 2005).

There is little doubt that use of low quality feed would be conducive for the development of biofouling assemblages when considered the production of nutrient and organic loading from feed wastage. Since it is not practical to control feed wastage in the water column of fish cage culture, improving and regulating feed quality such as the use of high quality fish feed may be the possible measure to reduce waste generated from fish feed input and possibly to reduce the development rates of biofouling assemblages. Several authors indicate that feed wastage and other waste output such as feacal material can be significantly reduced when high quality feed with better water stability, digestibility and optimal protein to energy ratio are used (e.g. Ove Arup, 1989; Hansen et al., 1990; Wu et al., 1994; Cho et al., 1994; Wu, 1995; Madin et al., 2009)

High quality fish feed are formulated to conform to certain characteristics so as to meet particular requirements of cultured fish (Nose & Halver, 1981). It generally has less bulk to store, uniform quality, and allow control over feed formulation (Lovell, 1993). The complete formulated diet supplies all the essential ingredients particularly protein, carbohydrates, fats, vitamins and minerals which are necessary for the optimal growth and health of the cultured fish (Harrison, 1990; Brown et al., 1997; Craig & Helfrich, 2002). Other important characteristics include good water stability, palatability, texture, friability, floatability, gelatinization, better selection of binders and

high digestibility level (e.g. Lim & Cuzon, 1994; De Silva & Anderson, 1995; Cuzon & Gehin, 1998; Devresse, 1998; Hillestad et al., 1999; Barrows, 2000; Sugiura & Hardy, 2000; Suresh & Zendejas, 2000; Dominy et al., 2001; Tacon & Obaldo, 2001; Kaushik, 2001).

There are three basic of pellets type available for aquaculture including compressed pellet, extruded dry pellet and semimoist extruded pellet (Booth at el., 2000; Craig & Helfrich, 2002). Extruded pellet are generally more stable in water as compared to non-extruded type because of smaller surface area exposed to the water. Seymour & Bergheim (1991) shows that extruded pellet remained up to 84% intact after 24 hour in water as compared 50% after 17 – 53 minutes for non extruded type in a study conducted for salmon feed. The higher water stability and digestibility of extruded pellets is able to reduce solid waste, nutrients loss due to leaching and produce high-energy diets for cultured fish (De Silva & Anderson, 1995; Hardy, 1999; Tacon, 2002). Several other benefits including the fact that it is less subject to fracture during shipping and handling, thus reduces the percentage of fines particle (Hardy, 1999).

The commercially-produced extruded fish feed is available in some Asian countries but they are expensive when compared to other types of formulated feeds owing to the high manufacturing costs (Boonyaratpalin, 1997). In Malaysia, the use of formulated commercial feed had been practiced long ago to feed grouper in floating net-cages (Chua & Teng, 1978; Teng et al., 1978), while extruded feeds are available since 1990s but their use is relatively lower as compared to trash fish feed due to the lack of information on its practical usage and the limited supply of ingredients (Musa & Nuruddin, 2005; Madin et al 2010).

The water parameter modification in the fish farm may inevitably combine with feed wastage to influence biofouling. Water current velocity and tidal regimes are

among the important factors influencing the colonization and succession of biofouling communities (Lewis, 1982; Baynes & Szmant, 1989; Riggio et al., 1986; Meyer-Reil & Koster, 2000; Madin et al., 2009; 2010). Other factors such as temperature, salinity and turbidity have been suggested to influence biofouling development (e.g. Bombace et al., 1994; Laihonen et al., 1996; Qvarfordt et al., 2006). The water parameters may vary depending on the fish farm location and possibly the net-cages position in the fish farm.

The specific objective of this study was to examine the effect of fish rearing, fish feed input, type of pellet feed, water flow attenuation and net-cage position in the fish farm on biofouling development on nets. This tested the hypothesis that fish rearing and thus the input of fish feed especially low quality feed i.e. home-made pellet increase biofouling rate. This is base on the finding that feed wastage and unassimilated food provide the additional organic matter for biofouling organisms (Chapter 3). It was also hypothesized that reduced current flow inside net-cages will enhance biofouling since the larvae and spores of biofoulers can settle and develop more successfully on the net, thus cage-nets located at the centre of the farm will foul up more quickly due to slower water flow. This study will also investigate the water flow attenuation due to the development of biofouling organisms on cage nettings.

4.2. RESULTS

4.2.1. Current Flow and Other Environmental Parameters

Experimental I

A 20% – 90% attenuation of water current velocity occurred as water flowed 10 m through the three serial, clean or unfouled fish cages at week 0 (Figure 4.1). However, the velocity of the water on encountering the first net cage (position 3) was reduced by 79% just after a week of immersion, and subsequently to as high as 91% reduction with further biofouling development. Flow attenuation also further increased to 89% as the

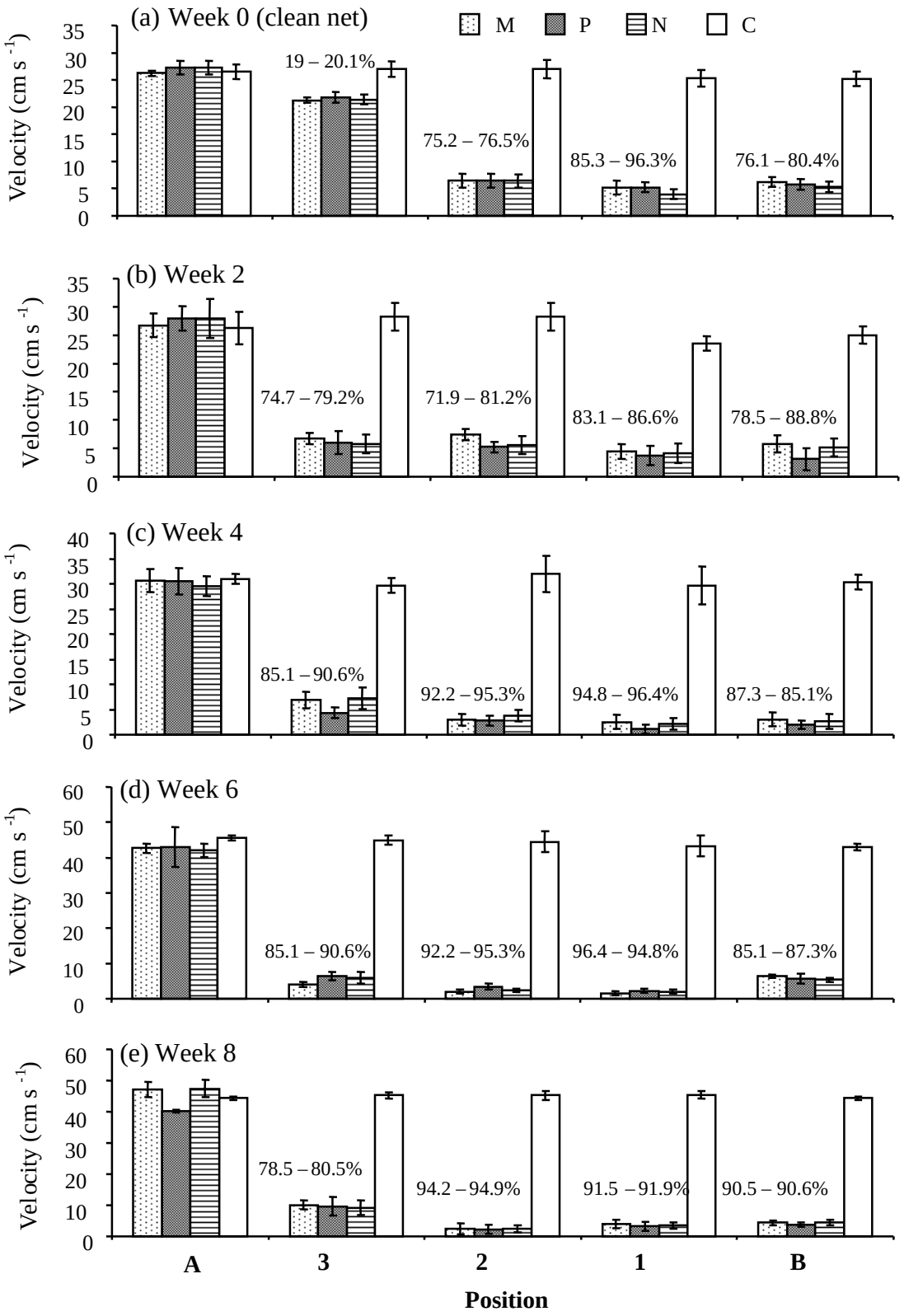


Figure 4.1. The fortnightly water flow velocity reduction (mean ± SD) from position A through net-cages 3, 2, 1 and position B of treatment M (commercially-produced extruded pellet feed), P (home-made pellet feed), N (without fish and feed inside net-cages) and C (without fish, feed and net-cages i.e. control) on (a) week 0 (clean net), (b) week 2, (c) week 4, (d) week 6 and (e) week 8 during the flood water in a floating fish farm at Jaha River estuary (Experiment I). See Figure 2.6a for details.

water flowed through the serial net-cages to position B at week 2. However, the flow attenuation only marginally increased (up to 91%) even at week 8. The results indicated that the physical presence of the net-cages themselves drastically obstructed water flow, as for instance, over 75% attenuation (i.e. at Position 2) was obtained after only passing through one clean net-cage unit. Outside the net-cages and in between the linearly arranged net-cages (A–C3–C2–C1–B), the water flowed unimpeded.

The effect of biofouling which reduced the flow rate by an additional 60% on week 2 and another 10% on week 4 was clearly obvious inside the first net (position 3), but not so in net-cages farther on the leeside where the water flow was so greatly reduced that the measured flow rates were not significantly different among each other ($P > 0.05$) (detailed data appended in Appendix 8).

Temperature, pH, salinity, dissolved oxygen and turbidity readings were not significantly different among net-cages given the different types of treatment, for both ebb and flood water (Table 4.1). However dissolved oxygen, salinity and temperature varied significantly ($P < 0.05$) between the flood and ebb water.

Experimental II

Surface flood flow velocities recorded along the transect D–I–U inside the farm (Stations S3 to S9) were significantly ($P < 0.001$) lower inside the net-cages (4.3 cm s^{-1}) than outside the net-cages (30.5 cm s^{-1}) (Figure 4.2a). However, the bottom velocity (39.6 cm s^{-1}) outside the net-cages but within the farm was the highest. This was also true during ebb flow through the farm, where surface velocity inside net-cages (3.6 cm s^{-1}) was the lowest, as compared to surface velocity (20 cm s^{-1}) and bottom velocity (26.3 cm s^{-1}) measured outside the net-cages (Figure 4.2b). Although the reduction in flow velocity on meeting the floating net-cage farm (i.e. at S3 and S9) was very drastic (up to 83%) inside the net-cages, there was surprisingly no further or very minimal

velocity attenuation as the water flowed 60 m further through the farm.

Table 4.1. Mean values of some environmental parameters recorded in a floating fish farm at Jaha River estuary during the (a) flood and (b) ebb water in Experiment I. Standard deviation (SD) in parentheses. Treatments, M (commercially-produced extruded pellet feed), P (home-made pellet feed), N (without feed and fish) and C (without feed, fish and net-cages).

a. Flood water

Treatments	M	P	N	C
pH	7.73 (0.09)	7.60 (0.15)	7.79 (0.10)	7.69 (0.10)
Temperature (ºC)	31.85 (0.89)*	31.72 (0.70)*	31.15 (0.43)*	31.76 (0.85)*
Salinity (ppt)	25.64 (0.16)*	23.36 (1.08)*	26.17 (0.11)*	26.06 (1.10)*
DO (mg l^{-1})	6.56 (2.20)*	5.74 (2.53)*	6.35 (0.73)*	6.23 (2.32)*
Turbidity (NTU)	78.39 (28.63)	81.15 (61.89)	75.23 (24.53)	81.60 (36.00)

b. Ebb water

Treatments	M	P	N	C
pH	7.13 (0.29)	7.09 (0.26)	7.22 (0.09)	7.08 (0.29)
Temperature (ºC)	31.28 (0.50)	30.88 (0.32)	30.94 (0.34)	30.94 (0.36)
Salinity (ppt)	23.83 (1.82)	22.27 (7.44)	21.24 (0.86)	23.06 (1.45)
DO (mg l^{-1})	2.91 (0.61)	2.99 (1.62)	2.50 (0.98)	2.37 (0.96)
Turbidity (NTU)	103.50 (16.63)	71.04 (48.12)	87.87 (13.79)	73.02 (23.25)

* indicate significance difference between flood and ebb water for factor ($P < 0.05$)

The various water parameters measured along the transect D–I–U varied significantly ($P < 0.05$) with tidal phase. Dissolved oxygen, salinity, temperature and pH decreased during ebb flow but turbidity increased. Along the transect, from flood to ebb flow, mean dissolved oxygen fell from 5.7 mg l^{-1} to 3 mg l^{-1}, salinity from 24.6 ppt to 23 ppt; temperature from 30.4 ^{o}C to 30.1 ^{o}C; pH from 7.6 to 7.1; but turbidity increased from 42.3 NTU to 87.9 NTU.

During flood tide, dissolved oxygen, turbidity and pH at station were significantly different ($P < 0.05$) among the three measured positions along the transect D–I–U. Mean surface dissolved oxygen, outside (6.4 mg l^{-1}) and inside (6.3 mg l^{-1}) the net-cages were

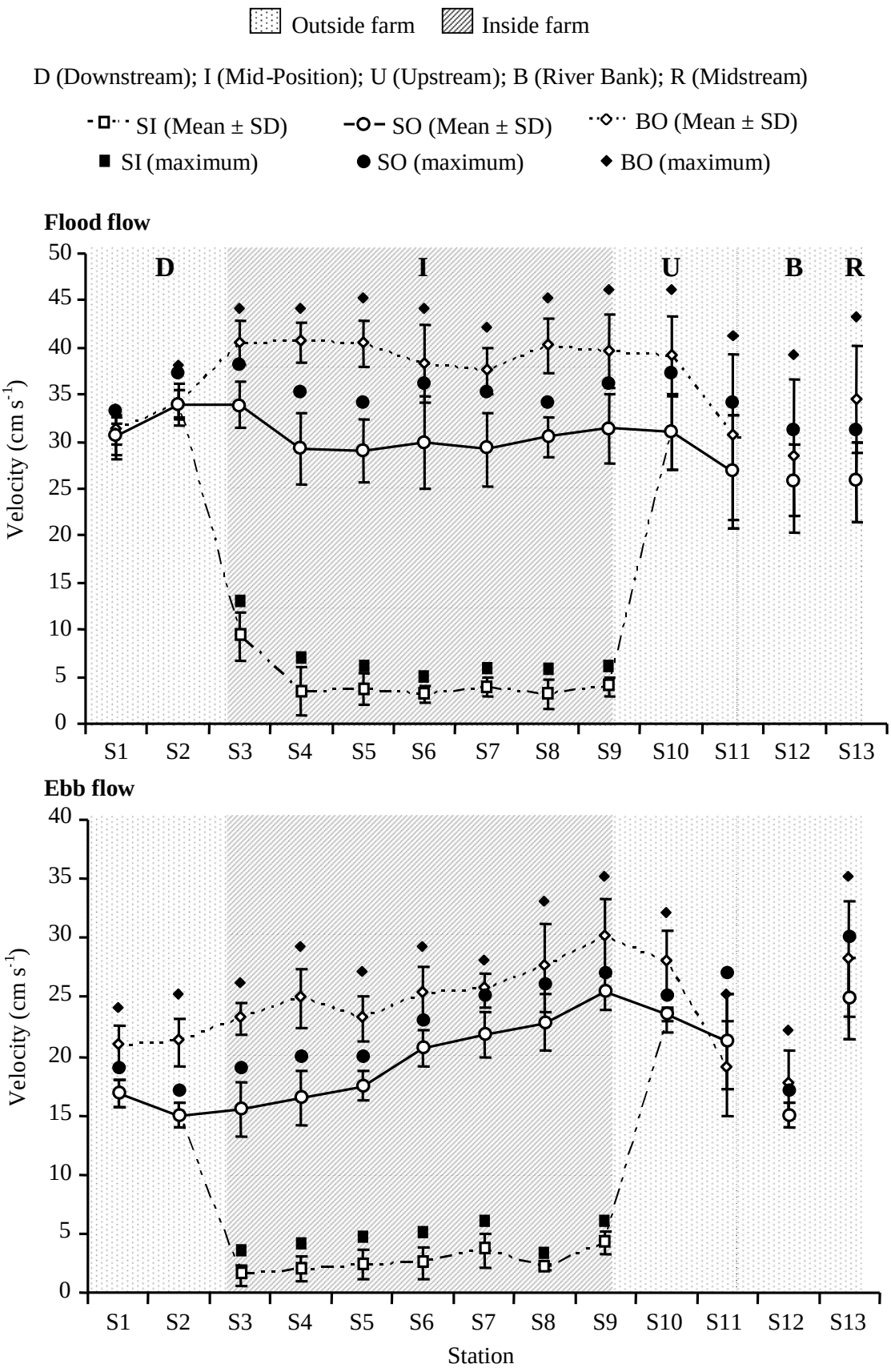


Figure 4.2. Mean (± SD) and maximum of flood and ebb tidal velocities (cm s^{-1}) recorded along transect D – I – U (Stations S1 – S11), S12 at the river bank (B) and S13 at midstream (R) (Experiment II). Measurements were made at the surface, inside net-cages (SI); surface, outside net-cages (SO) and bottom, outside net-cages (BO). See Figure 2.6b for details.

significantly higher than at the bottom (4.6 mg l^{-1}). Dissolved oxygen was high along stations S3 – S8 but dropped thereafter from stations S9 – S11 (Figure 4.3). Turbidity was significantly higher ($P < 0.05$) at the bottom water (36.7 NTU) as compared to the surface water, outside (28 NTU) or inside (28.1 NTU) net-cages. However, turbidity was generally similar along the transect line from stations S1 – S11. Other parameters such as temperature and salinity were however not significantly different ($P > 0.05$) among the three positions of measurements while pH was generally constant.

During ebb flow, dissolved oxygen, salinity, temperature, turbidity and pH at the three positions were however not significantly ($P > 0.05$) different. Unlike flood flow, the more turbid water on ebb tide deposited most of its suspended loadings as it traveled through the net-cage farm (see Figure 4.3).

4.2.2. Effect of Fish Rearing and Fish Feed on Biofouling Development (Experimental I)

The main effects of fish rearing, time (week) and their interaction were all very significant ($P < 0.01$) on sessile biofouling, explaining 29%, 60% and 7% of its total variability in biomass, respectively. Fouling biomass (g per 0.4 m^2 net panel dimension, or g per panel) was significantly higher inside (P, M, N) than outside (C) the cages, higher in feed-given (P, M) than no-feed (N, C) treatments, but with no significant difference between the two types of feed (P, M). The biofouling biomass increased steadily and significantly with time until week 6 and stabilized thereafter (Figure 4.4a, b). For non-sessile fouling, the main effects of feed, time and their interaction were similarly very significant, explaining 42%, 40% and 14% of the total biomass variability. The feed effect among treatments was exactly similar to sessile fouling. The time effect was also significant among weeks, except for the following homogenous groups (weeks): (1, 2), (4, 5) and (6, 7, 8) (Appendix 9).

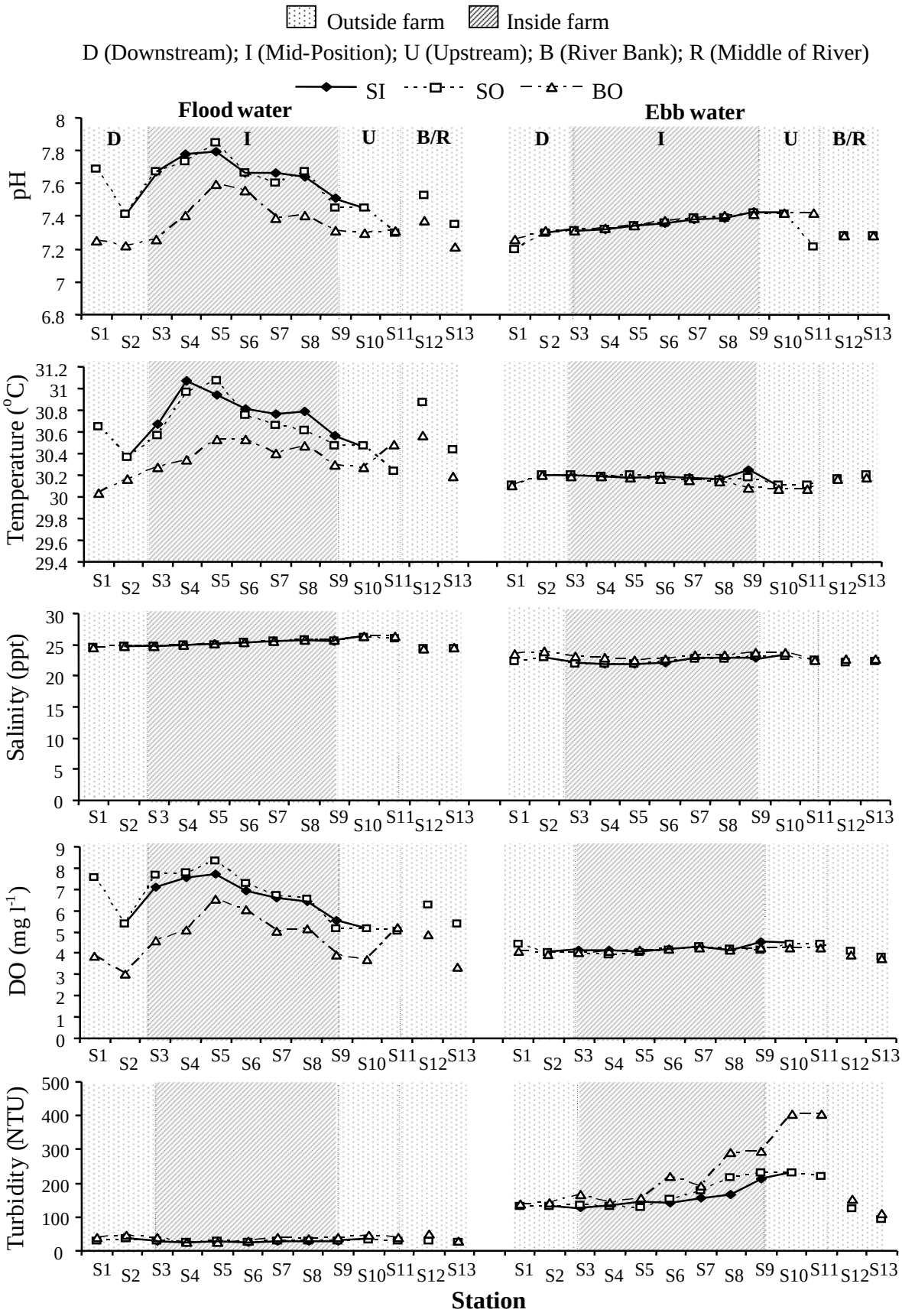
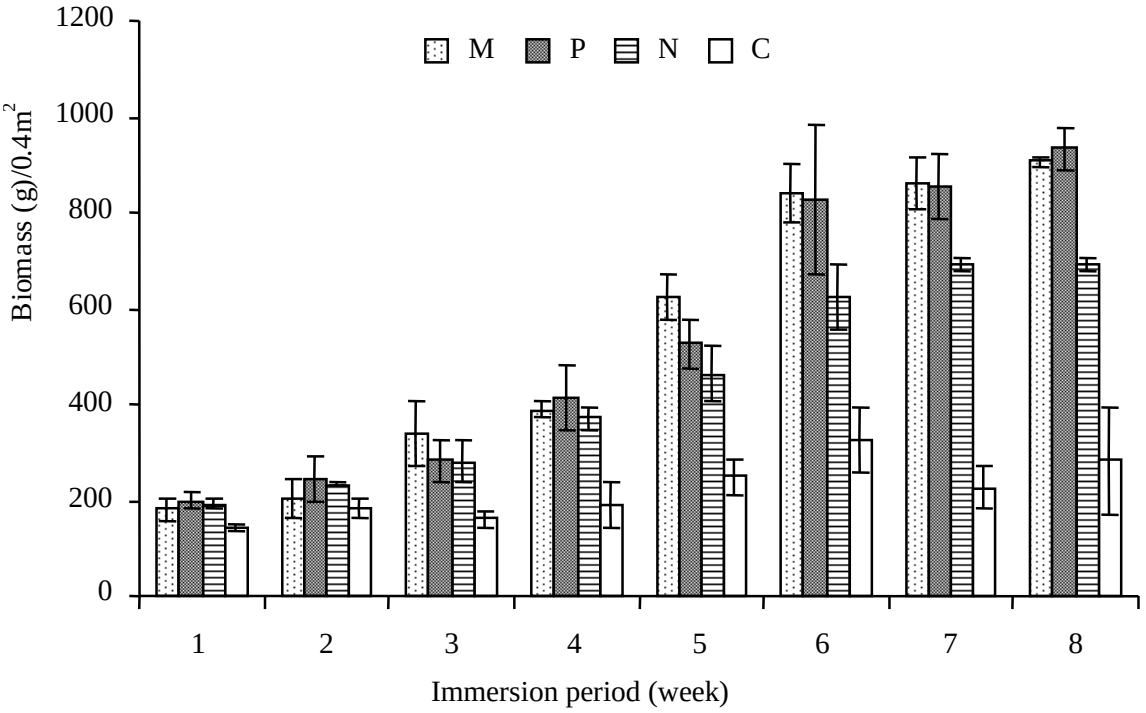
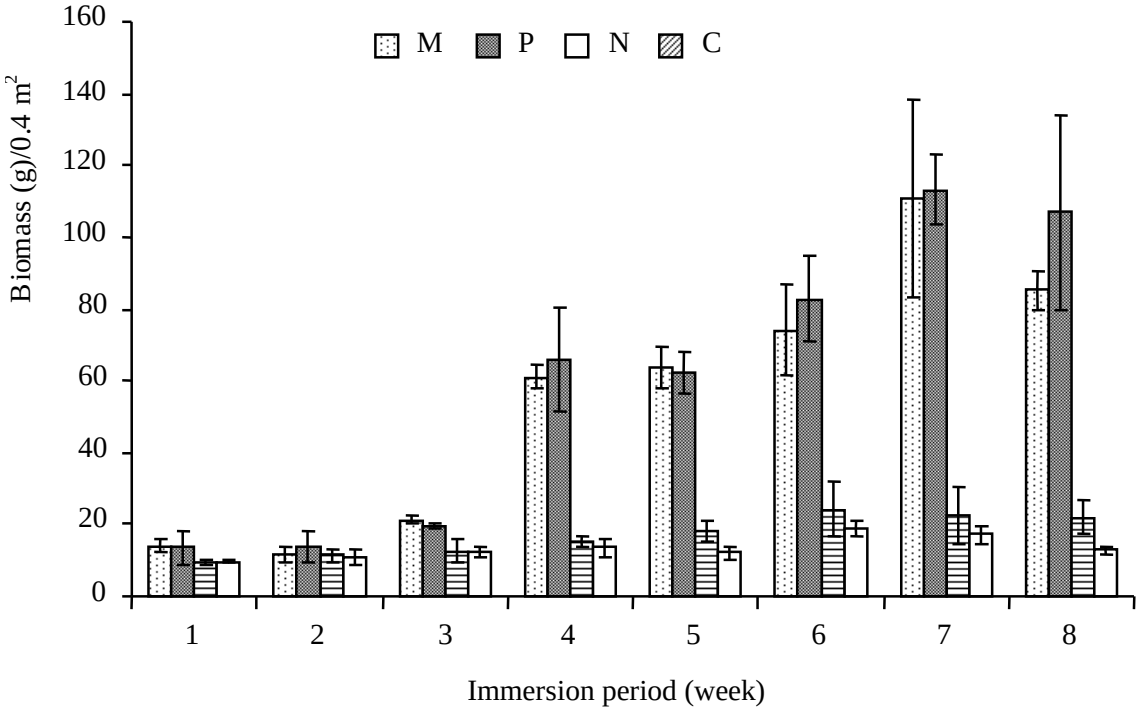


Figure 4.3. Mean pH, temperature, salinity, DO and turbidity, recorded along transect (S1 – S11), S12 at the river bank (B), and S13 at mid river (R). Measurements were made at surface, inside net-cages (SI); surface, outside net-cages (SO) and bottom, outside net-cages (BO), during the flood and ebb water in a floating fish farm at Jaha River estuary (Experiment II). See Figure 2.6b for details.



a. Sessile biofoulers



b. Non-sessile associates

Figure 4.4. Weekly biomass development of (a) sessile biofoulers and (b) non-sessile associates (mean ± SD) on net panels given the following treatments: M (commercially-produced extruded pellet feed), P (home-made pellet feed), N (without fish and feed) and outside the net-cages C (without fish and feed) in a floating fish farm at Jaha River estuary (Experiment I).

At the end of the 8th week, sessile biofouling on net panels for treatments M, P and N gave mean biomass (g per panel) of 906.1 g, 932.7 g and 691.4 g respectively, as compared to only 281.6 g for treatment C (see Figure 4.4a). Mean biomass (g per panel) of non-sessile organisms rapidly increased after the 3rd week, reaching highest value by the 7th week for treatments M (110.6 g) and P (113.1 g) (see Figure 4.4b). These values were significantly much higher than for the treatment N (22.2 g) and C (17 g).

The results indicate that the biomass of non-sessile organisms was significantly correlated to the biomass of sessile organisms inside M ($r = 0.85$), P ($r = 0.81$) and N ($r = 0.65$) net-cages as well as outside them, C ($r = 0.60$).

4.2.3. Effect of Fish Rearing, Fish Feed and Net-Cage Position on Biofouling Development (Experimental II)

The highest biomass (g per 0.4 m^2 net panel dimension, or g per panel) of sessile biofoulers on net panels was almost achieved by the 6th week for net-cages with fish rearing, i.e. those stocked with fish fed with either commercial feed pellet, M (707.8 g) or trash-fish, T (737.3 g), whereas for treatment N or without fish rearing, the fouling biomass was 40% less (464.9 g) (Table 4.2). Although there was significantly higher biomass from sessile biofouling in net-cages with fish rearing (M, T) as compared to unused net-cages (N) in the 1st week ($P < 0.05$), the difference was not significant for subsequent weeks ($P > 0.05$) based on the Latin Square ANOVA. The biomass of both sessile and non-sessile biofouling organisms on the net panels was also not significantly influenced by net-cage position throughout the 8 weeks of study, i.e. with respect to the longitudinal (D– I–U) and cross river (R–I–B) axes of the farm. Thus, the data were further analyzed using two-factor (feed and time) ANOVA with equal replication, after removing the "position" factor. The 2-factor ANOVA test thus provided more statistical power than the Latin Square ANOVA and was able to detect significantly higher fouling biomass in feed treatments M and T as compared to N (see Table 4.2).

Table 4.2. Weekly development in mean biomass (g per panel, mean ± SD) of sessile and non-sessile biofoulers in Experiment II. Net-cages with fish given commercially-produced pellet feed (M); net-cages with fish given trash-fish (T); and net-cages without fish and feed (N). Net-cage position along longitudinal from Downstream (D); Mid-position (I) and Upstream (U) and cross river from Mid-river (R); Mid-position (I) and River-bank (B). See Figure 2.6b for details.

	Week							
	1	2	3	4	5	6	7	8
Sessile								
M	219.0 ± 18.8 $^{*\Psi a}$	284.1 ± 79.9	250.2 ± 19.5	305.8 ± 31.4 $^{\Psi a}$	443.9 ± 115.9	707.8 ± 100.6 $^{\Psi a}$	791.1 ± 21.2	817.7 ± 100.1 $^{\Psi a}$
T	222.3 ± 19.1 $^{*\Psi a}$	287.6 ± 119.6	218.8 ± 37.7	294.5 ± 38.7 $^{\Psi a}$	519.6 ± 48.8	737.3 ± 121.3 $^{\Psi a}$	713.7 ± 252.0	777.2 ± 122.2 $^{\Psi a}$
N	176.4 ± 7.0 $^{*\Psi b}$	313.3 ± 59.2	314.4 ± 124.2	214.7 ± 34.7 $^{\Psi b}$	357.8 ± 39.5	464.9 ± 30.2 $^{\Psi b}$	498.3 ± 65.5	441.8 ± 135.4 $^{\Psi b}$
D	193.0 ± 13.5	242.2 ± 32.7	239.2 ± 60.3	261.4 ± 35.3	427.8 ± 126.2	615.6 ± 165.2	559.3 ± 205.4	618.2 ± 323.5
I	205.4 ± 32.2	283.1 ± 87.1	225.2 ± 37.7	262.9 ± 82.6	430.2 ± 57.8	637.9 ± 165.9	774.8 ± 201.1	707.3 ± 178.0
U	219.4 ± 31.6	359.8 ± 74.9	318.9 ± 107.9	290.7 ± 46.8	463.3 ± 128.8	657.1 ± 191.9	668.9 ± 140.8	711.0 ± 162.4
R	215.5 ± 33.2	290.6 ± 79.8	256.7 ± 45.9	259.4 ± 34.0	472.1 ± 79.4	673.5 ± 189.5	717.6 ± 267.4	642.3 ± 311.9
I	200.1 ± 17.0	249.8 ± 29.0	298.6 ± 132.1	287.6 ± 49.4	446.7 ± 126.2	543.8 ± 91.9	590.1 ± 191.8	635.8 ± 109.0
B	202.2 ± 33.7	344.6 ± 104.8	228.0 ± 31.8	268.1 ± 82.8	402.5 ± 105.3	693.3 ± 169.6	695.4 ± 122.7	758.5 ± 217.9
Non-sessile								
M	9.9 ± 1.2	20.0 ± 5.5 $^{\Psi a}$	55.2 ± 1.1	61.2 ± 11.2 $^{\Psi a}$	96.6 ± 7.0 $^{*\Psi a}$	78.5 ± 11.0 $^{*\Psi a}$	74.0 ± 10.5 $^{*\Psi a}$	114.5 ± 40.8 $^{\Psi a}$
T	8.9 ± 0.5	13.9 ± 1.7 $^{\Psi a}$	42.8 ± 32.4	54.1 ± 22.3 $^{\Psi a}$	87.9 ± 8.2 $^{*\Psi a}$	81.0 ± 11.8 $^{*\Psi a}$	88.9 ± 14.8 $^{*\Psi a}$	110.2 ± 25.3 $^{\Psi a}$
N	8.5 ± 0.2	9.5 ± 1.1 $^{\Psi b}$	10.8 ± 4.0	9.6 ± 0.5 $^{\Psi b}$	21.2 ± 12.9 $^{*\Psi b}$	26.7 ± 17.4 $^{*\Psi b}$	25.9 ± 8.1 $^{*\Psi b}$	20.5 ± 8.2 $^{\Psi b}$
D	9.1 ± 0.9	16.3 ± 5.8	24.4 ± 25.5	32.7 ± 20.5	73.9 ± 34.7	69.7 ± 20.1	64.2 ± 27.1	97.4 ± 70.5
I	9.8 ± 1.1	15.5 ± 7.8	36.4 ± 26.0	41.2 ± 30.3	63.9 ± 43.3	59.5 ± 36.6	65.4 ± 37.6	92.4 ± 54.3
U	8.5 ± 0.1	11.7 ± 2.2	48.0 ± 29.9	51.0 ± 37.1	67.9 ± 47.1	56.9 ± 38.7	59.2 ± 38.1	55.4 ± 36.2
R	8.8 ± 0.5	12.7 ± 1.7	37.6 ± 23.8	38.8 ± 27.4	71.7 ± 30.9	75.7 ± 25.3	67.7 ± 33.8	72.2 ± 54.8
I	9.4 ± 1.4	16.3 ± 7.4	26.6 ± 25.7	39.8 ± 30.8	62.1 ± 42.4	54.2 ± 35.9	52.8 ± 30.5	85.3 ± 61.7
B	9.1 ± 0.9	14.4 ± 7.1	44.7 ± 34.1	46.3 ± 34.9	71.9 ± 50.2	56.3 ± 32.0	68.3 ± 36.4	87.8 ± 61.5

$^{a, b}$ *Indicates homogenous group*

* *Indicates significant different amongst treatments feed (M, T, N) for factor ($P < 0.05$), based on Latin Square Design ANOVA test*

$^{\Psi}$ *Indicates significant different amongst treatments feed (M, T, N) for factor ($P < 0.05$), based on two-way ANOVA test after removing the "position" factor*

These were observed in four and six weeks out of eight weeks for sessile and non-sessile biofouling respectively (detailed data appended in Appendix 10).

4.3. DISCUSSION

4.3.1. Effects of Fish Rearing and Fish Feed

It has been hypothesized (Chapter 3) that use of higher quality pellet feed including better water stability and vigorous texture will reduce fish feed wastage and thus the development of biofouling assemblages. However, in this study the biomass of especially sessile biofoulers given commercially-produced extruded pellet feed (M) was not significantly different ($P > 0.05$) to that given trash-fish feed (T) in Experiment II. This indicates that biofouling development was not influenced by the type or the quality of fish feed. This contention is also supported by results obtained from Experiment I, where there were no significant ($P > 0.05$) differences in mean biomass of both biofouling types between home-made pellet (P) and commercially-produced extruded pellet feed (M). This further indicates that even a high quality feed with better water stability as in commercially-produced extruded pellet feed did not help to reduce biofouling on nets. Therefore, the proposed hypothesis was not accepted.

Although fouling biomass was not influenced by the type of fish feed input, use of low quality feed such as trash fish feed had been proven to cause several negative implications on water quality of fish farm environment due to production of organic and solid waste (e.g. Cho et al., 1994; Wu, 1995; Islam, 2005). It has been recommended that the use of unprocessed feed such as trash fish needs to change and be replaced by industrially prepared feeds so as to avoid introducing potential diseases to cultured fish and into the environment (e.g. ADB/NACA, 1998; Gill, 2000; Tacon & Forster, 2000; Fegan, 2001; Prior et al., 2001; Rodrigues et al., 2001). Furthermore, the improperly

processed trash fish feed probably contains the spores of biofouling organisms that would increase the biofouling population inside the net-cages.

Fish rearing with fish feed input in floating net-cages significantly increase the biomass (g per panel) of biofouling on net panels. In feed receiving net-cages (whether M, P and T) non-sessile rates increased up to 80% higher after three weeks of development whereas sessile biofouling rates increased nearly 25% times higher after four weeks of development; as compared to net-cages without fish rearing 'N' (see Figure 4.4 or Table 4.2). The type of food essential for biofouling organisms are however difficult to assess, e.g. whether they consume fish feed wastage or rely on fish faeces or metabolite waste of cultivated fish. Furthermore, higher water stability of commercially-produced extruded pellet feed (M) which is expected to give a lower amount of feed wastage had similarly contributed to the higher biomass of biofoulers. This strongly indicates that fish faeces and other metabolite waste including excretion products of cultured fish may be important in enhancing the development of biofouling organisms. Excretion products of cultured fish consists mainly of nutrient such as ammonia released through the gills and phosphorus excreted via the urine and are present in both soluble and organic particulate form (Doglioli et al., 2004). Their release into the water column, along with the retarded water flow inside the net-cages could encourage the growth of algal fouling as well as phytoplankton blooms which provide food for suspension feeders.

According to Dudley et al. (2000), 1.9 g of faeces is produced for every kg of fishes cultured, while Vita et al. (2004) indicate that about 80% of the particulate organic matter and metabolic waste in a fish farm are produced by cultivated fish. Gowen & Bradbury (1987) noted that the amount of faecal waste is relatively higher in the immediate vicinity of fish farm. In the present study, high density of cultured fish (i.e. approximately 200 fishes per 9.38 m^3 net-cages dimension) and with retarded water

flow inside the net-cages (treatment M & P) would generate a high amount of fish faeces and other metabolite wastes that contribute to organic enrichment especially detritus for non-sessile organisms. Several kinds of detritus are essential component for the diet of aquatic fauna including zooplankton in estuaries (e.g. Pearson & Rosenberg, 1978; 1987; Edgar, 1990; Snelgrove & Butman, 1994; Grindley, 1981; Roman et al., 1983; Toner, 1991; Daniel & Potter, 1995). Furthermore, net-cage culture is an open system where waste material are inevitably released and attracts marine fauna owing to the higher availability of food particles within and around it (e.g. Kilambi et al., 1978, Dubost et al., 1998; Coasta-Pierce & Bridger, 2002; Ross et al., 2004).

In the present study, higher biofouling rate in cages given pelleted feed (M and P), similar to that of trash fish feed, is probably due to fishmeal ingredients which encourage the development of particularly the non-sessile scavengers. Fish feed ingredient of animal origin such as fish meal and other poultry byproduct meal contain high amounts of phosphorus that will also increase the amount of excreted phosphorus (Persson, 1991; De Silva & Anderson, 1995; Sugiura et al., 2000). This suggests that fish meal from pelleted feed (M and P) and trash fish feed similarly contribute to increased phosphorus concentration inside the net-cages. Alongi et al., (2003) had reported higher concentrations of PO_4^3 inside net-cages compared to non-cages sites in a fish farm at Jaha River estuary where trash fish feed is the common practice of fish feeding.

Boonyaratpalin & Phongmaneerat (1990) indicated that total phosphorus required by cultured fish such as seabass is relatively minor, at 0.55 – 0.65%. Therefore it is possible to improve fish feed formulation by reducing fish meal ingredients and thus the phosphorus loading and biofouling rates on cages nettings. Nevertheless, phosphorus nutrition is among the critical aspects in feed formulation and selecting a feed ingredient with high phosphorus bioavailability by adding phytase and reduce the amount of

insoluble phosphorus is important (Ketola, 1985; Cain & Garling, 1995). Thus, use of plant ingredient such as soybean and palm oil for inclusion in fish feed had been suggested to be an alternative to reduce phosphorus from fish meal ingredients (e.g. Tacon et al., 1983; Hardy et al., 1987; Kaushik, 1990; Watanabe & Pongmaneerat, 1993; Kaushik et al., 1995; Torstensen et al., 2000; Gunstone, 2001; Bell et al., 2002; Regost et al., 2002).

4.3.2. Effects of Water Flow

The water flow attenuation through an unfouled net-cage of 16 mm mesh size was approximately 20% which is comparable to a 25% flow reduction through clean nets of 12 – 18 mm mesh sizes observed by Cook (2001). With progressive biofouling, as high as 90% flow velocity diminution was obtained in this study after 3 weeks of biofouling. As a result of impedance, the water flow on encountering the floating net-cage farm was significantly deflected to below the net-cages, thereby increasing the flow velocity here (see Figure 4.1). This mode of deflection explains why all net-cages further into the leeside experienced very similar but weak flow to the extent that even net fouling did not significantly reduce the flow rate any further. The result indicates that biofouling development on cage netting, as well as the net-cage units themselves (without biofouling) will significantly cause water flow reduction inside the net-cages.

According to Black (1998), water movement is essential in net-cage culture for the removal of waste products. The reduction of water movements that remove uneaten fish feed, fish faeces and nutrients from cultured fish cages could enhance the growth of biofouling organisms. This is substantiated by Experiment I, where net panels placed outside the net-cages (i.e. treatment C), subjected to stronger flow rate (>20 cm s^{-1}) and without the benefit of fish feed, carried much less biofouling. The relative effect of water flow and its interaction with food availability on sessile and non-sessile biofoulers

inside the net-cages is however difficult to assess. It is appears that flow rate is more critical than feed input because irrespective of the amount of feed given, swifter current flow will make less available the food to biofoulers on net panels (Madin et al., 2010).

The present study (Experiment I) suggests that if the flow rate through net-cages is slow (<10 cm s^{-1}), biofouling biomass (g per panel) by sessile organisms will be significantly increased. For example, their rates were nearly 50% higher in treatment N (without fish rearing and feed) and nearly 65% higher in treatment M and P (with fish rearing and feed) compared to treatment outside net-cages 'C' (swifter water flow i.e. >25 cm s^{-1}) after four weeks of development (see Figure 4.4a). This suggests that swifter water flow (i.e. >25 cm s^{-1}) and low organic input could significantly reduce sessile biofouling by almost 65%. Unlike the sessile organisms, non-sessile organisms were more attracted to the organic inputs from fish rearing inside the net-cages rather than the reduced water flow rate. For example, their biomass (g per panel) rates in treatments N (slow water flow i.e. <10 cm s^{-1}) and C (swifter water flow i.e. >25 cm s^{-1}) was about an equal that it is nearly 80 – 85% lower than in fish rearing net-cages (whether M and P with slow water flow i.e. <10 cm s^{-1}) after three weeks of development (see Figure 4.4b) (see also Madin et al., 2010).

Results from Experiment I indicate that a flow velocity of 25 cm s^{-1} is probably near to the critical threshold below which biofouling rapidly develops. For instance, a 20% flow reduction (i.e. flow velocity of ca. 20 cm s^{-1}) in the clean nets of frontline net-cages (net position 3, Figure 4.1) had initiated rapid biofouling which further reduced the flow velocity by 75 – 80% (<10 cm s^{-1}) after two weeks of immersion. This drastic velocity reduction after two weeks of immersion apparently sets the pace for rapid sessile biofouling in the subsequent weeks (see Figure 4.4). On the other hand, outside the fish cage where the flow rate was high (>25 cm s^{-1}), the net biofouling rate remained low and constant over weeks.

The rate of water flow can have a significant impact on the distribution, structural form and feeding behavior of aquatic organisms (e.g. Wildish & Kristmanson, 1997; Zhang & Malmqvist, 1997; Leonard et al., 1998). In the present study, stress from strong water flow experienced by sessile organisms outside the net-cages could reduce settlement, growth rate and development resulting in lower mean biomass (see also Madin et al., 2010). They may experience drag forces that required high flexibility of stolon to support their growth and avoid detachment from strong water flow. A number of study show similar implication of strong water flow on the development of sessile organisms, for example, Ryder et al. (2004) noted that strong water flow (>13.7 cm s^{-1}) could reduce the growth rate of thallus and spore development of *Gracilaria*. In strong water flow or under wave exposed habitat, marine invertebrates such as barnacles are shorter and bear thicker shells to resist detachment (Pentcheff, 1995; Arsenault et al., 2001), while mussels have thicker shells and reduced height to width ratio to avoid detachment (Raubenheimer & Cook, 1990; Akester & Martel, 2000).

Water flow rates will significantly influence the feeding performance of suspension feeders since they depends on current flow to obtain their food (e.g. Okamura, 1984; 1985; Cheer & Koehl, 1987; Zhang & Malmqvist, 1997; Okamura & Partridge, 1999). According to Eckman & Duggins (1993) and Okamura & Partridge (1999), optimal flow for feeding exists for many suspensions feeder species; it will be suppressed when flow is too fast or too slow. In the present study, retarded water flow (<10 cm s^{-1}) as occurring inside the net-cages is likely to be an optimal for the feeding of suspension feeder macrofoulers which exploit production from the water column including phytoplankton and nutrients. Slower flow rates will give them more time to filter more foods as it is in contact with water column for longer periods while food depletion could occurs outside the net-cages and they have to compete for the fast moving food.

Spore and larval behavior of biofoulers including their settlement and final dispersal

are also influenced by water flow rates (e.g. Sulkin, 1981; Stancyk & Feller, 1986; Wethey, 1984; 1986; Mullineaux & Butman, 1990; Young, 1995; Underwood & Keough, 2001; Madin et al., 2009). In the present study, slower flow rates inside the net-cages could encourage settlement of biofouling larval and spore. They can easily attach and develop, while their residence times are sufficiently long to reach competency to colonize the net panels. Their subsequent development on net panels appears to be little affected by fish rearing (e.g. without fish feed input and cultivated fish). Apparently, once established the sessile colonizers could live on the available suspended organic matter or without (e.g. seaweeds).

Although biofouling organisms such as barnacles and hydroids can double their recruitment and settlement in fast flowing water (e.g. Bertness et al., 1991), in the present study, more biofouling organisms when water flow is reduced as was inside the net-cages. Qian et al. (2000) noted that larval settlement of barnacles was at its highest if flow rates are between 2.1 cm s^{-1} – 10.6 cm s^{-1} and did not settle when the flow rate over 21 cm s^{-1}. This is consistent with the present finding (Chapter 3), where population of *B. amphitrite* were significantly higher inside the net-cages of slow water flow (<10 cm s^{-1}) than outside it (> 25 cm s^{-1}).

4.3.3. Effect of Net-Cage Position and Other Factors

The development rates of biofouling assemblages were not significantly influenced by the net-cage position in the fish farms. Mean total wet biomass of sessile and non-sessile biofoulers, and their weekly development rates were relatively similar along the longitudinal (D–I–U) and cross-river (R–I–B) direction, which indicates that the slow current flow and food availability inside the net-cages are rather consistent in the fish farm. Results from Experiment II, show that current flow along transect station (S3 – S9) was relatively slower (<10 cm s^{-1}) inside the net-cages as compared to the much

stronger water flow (>20 cm s^{-1}) recorded outside them (see Figure 4.2).

In the present study, although the reduction of flow velocity on meeting the floating net-cage farm (i.e. flood flow at S3 or ebb flow at S9) was very drastic (up to 15 times) inside the net-cages, there was surprisingly no further velocity attenuation as the water passed further through the farm (60 m). However, stronger water flow below and sideway along the unrestricted space beneath the net-cages maybe important to disperse and wash out the waste material from fish cages. Poor flushing can cause environmental damage especially in the immediate vicinity of fish farm (Goldburg et al., 1996).

The other water parameters such as salinity, dissolved oxygen, and turbidity along transect were relatively different between ebb and flood tidal flows, stations and position (outside vs. inside cages) at station. This could influence the development of biofouling assemblages throughout the fish farm. For example, slightly higher biomass of sessile biofoulers at the upstream end of th farm (i.e. station S8 – S11) could also due to higher water turbidity (200 – 400 NTU) during ebb water (see Table 4.2 and Figure 4.3). Increased turbidity had been suggested to influence the succession of biofouling communities.

The concentration of dissolved oxygen varied significantly during the ebb and flood water as well as between the inside and outside net-cages stations. The aquaculture activity is deemed responsible for the oxygen depletion inside the fish farm due to consumption by cultured fish and other organisms including the biofoulers. Other factors such as redox processes during the degradation of sinking organic waste are also thought to cause oxygen depletion (Tovar et al., 2000).

4.4. CONCLUSION

The study concludes that the physical presence of the floating net-cages and biofouling of cage nettings reduce water flow through them by as much as 20% and 70%

respectively. The first factor is responsible for the drastic reduction of water flow through serial net cages on the leeward side resulting in consistently low flow velocity across the farm. Biofouling rates are thus high and consistent for serial net-cages across the farm, both longitudinally and transversely. The use of quality pellet feed vis-à-vis trash-fish feed will not reduce both sessile and non-sessile biofouling on cage nettings. Thus, the proposed hypothesis that fish feed with high water stability and less fine particulate content contributes to lower biofouling than fish feed of low water stability and high particulate content was not accepted. Based on the result of present study, total biofouling rate outside the net-cage where water flow was swifter (>25 cm s^{-1}) is estimated at 92 g m^{-2} wk^{-1}, but reduced water flow (<10 cm s^{-1}) inside the net-cages could increase the biofouling rate to 223 g m^{-2} wk^{-1} (142%) attributable to the higher contribution of sessile biofoulers. Fish rearing and thus fish feed input, fish faeces and other metabolite waste of cultivated fish in a flow-reduced situation further increased the biofouling rate to 310 g m^{-2} wk^{-1} (237%) attributable to the higher contribution of sessile and non-sessile biofoulers respectively (Table 4.3).

Table 4.3. Summarized data of highest (i.e. on 8^{th} week) mean biomass of sessile and non-sessile biofouling organisms among treatment feed in Experiment I (C, N, M, P) and Experiment II (N, T, M). Submersion period in weeks is given in parentheses. Mean biofouling rate based on total weight (g) on 1 m^2 of net panel per week.

	Biofouling rates (g m^{-2})			Mean rate (g m^{-2} wk^{-1})
	Sessile	Non-sessile	Total	
Experiment I				
No fish, feed and enclosing cage-netting, (C)	704	31.7	735.7	92
Net-cage without fish and feed, (N)	1728.5	54.5	1783	223
Fish given commercially-produced extruded pellet feed, (M)	2265.2	212.7	2477.9	310
Stocked fish fed home-made pellet feed, (P)	2331.7	267	2598.7	325
Experiment II				
Net-cage without fish and feed, (N)	1104.5	51.2	1155.7	145
Stocked fish fed trash-fish feed, (T)	1943	275.5	2217.5	277
Stocked fish fed commercially-produced extruded pellet feed, (M)	2044.2	286.2	2327.5	291

CHAPTER 5

EFFECTS OF SALINITY ON MACROFOULING COMMUNITY STRUCTURE

Summary of Important Finding

The proposed hypothesis that higher salinity (i.e. > 20 ppt) increases the biofouling rates on cage nettings cannot be generalized for the different macrofouling organisms. Percentage cover of anthozoans (unidentified anemone) and macroalgae (*Polysiphonia* sp.) was significantly reduced when subjected to low salinity (i.e. < 15 ppt) while high salinity (i.e. > 20 ppt) seems to increase their population. The cover of hydroid (*Plumularia* sp.), barnacles (*Balanus amphitrite*) and bryozoans (*Cryptosula* sp.) were relatively constant or increased slightly irrespective of the salinity, while for *Enteromorpha clathrata* and mussel (*Xenostrobus mangle*), cover decreased at all salinities. However, most of the studied macrofoulers survived at least for a week or for three weeks, indicating their ability to tolerate a wide range of salinity and therefore they are prevalent foulers of floating net-cages in estuarine waters.

5.1. INTRODUCTION

Salinity is well known to influence the development of biofouling organisms, with an optimal range that favors their development (e.g. Qiu et al., 1997; 1998; Underwood & Keough, 2001; Witman & Dayton, 2001). Based on results reported in Chapter 3, relatively higher salinity in the estuary (i.e. 24.01 – 25.07 ppt during the dry season) seems to enhance the development rates of several macrofoulers such as *Polysiphonia* sp., *E. clathrata*, sea anemone, *B. amphitrite*, amphipods and tanaids. Their growth rates and thus their cover were almost two times higher during the dry season than in the wet season which had a lower salinity (i.e. 15.17 –18.47 ppt). In the wet season, the abundance of the dominant 'dry season' species was substantially reduced, but the low salinity condition is likely favored by *Plumularia* sp., *X. mangle*, nematode and

copepods. Their growth rates and thus their cover increased significantly during the wet season. It was thus hypothesized that higher salinity (as observed during the dry season) is suitable for marine biofouling forms thus increasing their biofouling rates. Conversely, low salinity decreases the biofouling rate and thus gives a lower biofouling biomass. Thus, one of the objectives of this chapter was to test the proposed hypothesis.

However, the range within which salinity effects are more pronounced on biofouling organisms is difficult to assess since it depends on species-specific tolerance. In addition, the ability of organisms to tolerate a wide range of salinity fluctuations as frequently occurring in the estuary is also difficult to determine, including the optimal salinity range preferred by these biofouling organisms. Higher salinity is likely to provide a conducive environment for the growth of marine species adapted to live in an estuary and thus depends on their capability to tolerate salinity fluctuation. Therefore, another purpose of this study was to determine the preferred salinity range for the development of various species of biofouling organisms.

Capurro (1970) defines salinity as "the total of solid materials in grams in 1 kg of sea water when all the carbonate has been converted to oxide, the bromine and iodine replaced by chlorine, and all organic material completely oxidized". Salinity is non-toxic inorganic constituent but may cause toxic effects at extreme concentrations (DWAF, 1996; Leske & Buckley, 2003). Salinity in the open sea is relatively stable at 35 ppt (Pickard & Emery, 1990) therefore marine organisms have no difficulty to survive. However, salinity fluctuations occurred regularly and unpredictably in the estuary due to inflows of freshwater and saltwater, ambient heating, cooling as seasonal weather conditions change, mixing by currents, tidal inundation, etc. (e.g. Pickard & Emery, 1990; Digby et al., 1998; IPCC, 2001).

Kennish (1986) quotes Pritchard (1967) in defining the estuary as a semi-enclosed

coastal body of water which has a free connection with the open sea and within which seawater is diluted with freshwater. Decrease in salinity is due to freshwater input from river or rain water being added, while increase in salinity is due to a removal of fresh water which occurs via evaporation and decreased input of freshwater. Freshwater inputs can significantly modify the level of salinity where the lower salinity always coincided with rainfall and fresh water run-off during the rainy season (e.g. Michie et al., 1991; Kitheka, 1996; Osore et al., 1997; Padovan, 1997; 2003). Unlike the salinity of estuarine water in the temperate region, salinity in tropical region can undergo fast and important changes due to monsoonal regimes (Lazareth et al., 2003).

Salinity can have significant effects on ecological and biological processes including metabolic rates, nutrient cycling, community composition, colonization, seed germination and species richness (e.g. Kinne, 1964; Mason, 1986; Jones, 1988; Montague & Ley, 1993; Dallas et al., 1998; Nielson et al., 2003). For example, species diversity is relatively low in estuaries due to the large salinity fluctuations causing physiological stress that negatively affect the growth and survival of a wide range of marine organisms (Pechenik, 1987; Tedengren, et al., 1988; Greger & Kautsky, 1991; Pechenik et al., 1998; Kautsky, 1998; Roy et al., 2001). Moreover, capabilities to tolerate wide fluctuations in salinity often play a crucial role in the development of stable populations as well as their distribution (Sokolova, 2000; Hedgpeth, 1983).

Tolerance to the wide fluctuations in salinity requires adjustment or adaptation of species (e.g. McLusky, 1971; Savage, 1981; Williams & Williams, 1998). The mobile and sessile organisms respond differently to the salinity fluctuation. For example, zooplankton dynamics in estuarine water are regulated by salinity and behavioral pattern in swimming or migration in response to changes in salinity is one of their vital adaptations (e.g. Dekshenieks et al., 1996; Wooldridge & Callahan, 2000). Sessile

organisms including plants and animals have effective osmo-regulatory systems to adapt to salinity fluctuation (e.g. Lobban & Harrison, 1994; Fu & Bell, 2003).

Salinity interacts with other water parameters as well as other physical and chemical factors such as pollution, substrate types etc. that directly affect the distribution of estuarine organisms (e.g. Dethier & Schoch, 2005). For example, salinity and its interaction with other parameters such as dissolved oxygen within normal ranges could cause stress or mortality to marine organisms particularly the benthic invertebrates (Culter, 1997). Combined effects of salinity and anthropogenic activities such as pollution had been suggested to influence biofouling successions (Mayer-Pinto & Junqueira, 2003) as well as the community structure of organisms in the estuary (Kennish, 1990; Gaston et al., 1998; Venturini et al., 2003).

The present study was carried out in order to acquire a better understanding of how seasonal salinity fluctuations in estuarine water influence the community structure of sessile macrofouling organisms. The experiment was carried out by studying the development/growth and survival rates of several sessile macrofouling species when subjected to different salinities in the laboratory.

The specific objective of this study was to test the hypothesis that higher salinity as observed during the dry season is more suitable for marine biofouling forms thus increasing biofouling rates on cage nettings. Conversely, low salinity as observed during the wet season decreases the biofouling rate because the fast-growing species in higher salinity are able to tolerate lower salinity. To test the above hypothesis, the study will determine survival of selected biofouling organisms to salinity that ranged between 10 – 30 ppt, the observed range that was recorded in the study area. The survival was measured by percent cover of live organisms.

5.2. RESULTS

5.2.1. Macrofouling Community Structure in 10 ppt

Percentage cover of anthozoans and *Polysiphonia* sp. was significantly reduced when subjected to 10 ppt treatment. Cover of anthozoans was significantly ($P < 0.05$) reduced from 41.42% at week 0 to 14% at week 3, while cover of *Polysiphonia* sp. was significantly ($P < 0.05$) reduced from 52.33% at week 0 to 7.26% at week 3 (Table 5.1).

Table 5.1. Weekly changes in percentage cover (%) of macrofouling organisms in 10 ppt. Standard deviation (SD) in parentheses.

	Week			
	0	**1**	**2**	**3**
Plumularia sp.	58.27 (33.58)	59.79 (24.13)	59.51 (24.28)	62.23 (18.01)
Anthozoans	41.42 (39.46)	28.09 (25.96)	16.49 (16.63)	14.00 (17.95)*
B. amphitrite	24.14 (25.46)	23.34 (25.53)	28.08 (23.77)	24.44 (25.23)
Polysiphonia sp.	52.33 (43.52)	34.66 (31.34)	5.75 (11.91)	7.26 (13.23)*
E. clathrata	50.89 (47.49)	26.65 (23.15)	11.75 (15.36)	10.71 (14.01)
X. mangle	12.82 (3.85)	12.82 (3.85)	0.00 (0.00)	0.00 (0.00)
Cryptosula sp.	47.42 (42.88)	34.19 (33.16)	7.61 (9.92)	10.77 (14.04)

* *Indicates significant (P<0.05) difference between the initial percentage cover at week 0*

There were relatively no significant ($P > 0.05$) reductions or increments in percentage cover of other macrofoulers. For example, *Plumularia* sp. and *B. amphitrite* was constant from week 0 until the 3rd week of experiment, while *E. clathrata*, *Cryptosula* sp. and *X. mangle* decreased (detailed data appended in Appendix 11).

5.2.2. Macrofouling Community Structure in 15 ppt

Similar to result of 10 ppt, percentage cover of anthozoans and *Polysiphonia* sp. was significantly reduced in 15 ppt. Cover of anthozoans significantly ($P < 0.05$) reduced from 33.72% at week 0 to 15.21% at week 2 while covers of *Polysiphonia* sp. significantly ($P < 0.05$) reduced or disappeared at the 3rd week from an initial cover of 78.35% at week 0 (Table 5.2) (see Appendix 11).

Table 5.2. Weekly changes in percentage cover (%) of sessile macrofouling organisms in 15 ppt. Standard deviation (SD) in parentheses.

	Week			
	0	**1**	**2**	**3**
Plumularia sp.	47.71 (29.84)	44.19 (23.92)	41.09 (23.28)	41.89 (22.95)
Anthozoans	33.72 (25.87)	24.01 (25.44)	15.21 (20.77)*	27.05 (27.92)
B. amphitrite	15.19 (24.74)	15.19 (25.17)	34.81 (24.11)	34.81 (25.98)
Polysiphonia sp.	78.36 (69.71)	21.64 (30.29)*	0.00 (0.00)	0.00 (0.00)
E. clathrata	45.15 (44.10)	29.99 (28.51)	15.52 (17.05)	9.33 (10.34)
X. mangle	15.84 (2.54)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Cryptosula sp.	0.00 (0.00)	22.38 (22.38)	40.99 (40.99)	36.63 (36.63)

* *Indicates significant (P<0.05) difference between the initial percentage cover at week 0*

There were no significant ($P > 0.05$) reductions or increment in the percentage cover of other macrofoulers. Covers of *Plumularia* sp. were relatively constant at 41 – 48% from week 0 until the week 3, while *B. amphitrite* and *Cryptosula* sp. slightly increased. The initial percentage cover (45.15%) of *E. clathrata* at week 0 gradually decreased to 9.33% at week 3 while *X. mangle* with initial cover of 15.84% at week 0 totally disappeared after 1 week of experiment.

5.2.3. Macrofouling Community Structure in 20 ppt

There were relatively no significant ($P > 0.05$) increments or reduction in the percentage cover of macrofouling organisms when subjected to 20 ppt. Covers of *Plumularia* sp. and anthozoans were relatively constant or increased slightly from week 0 until the 3rd week of experiment (Table 5.3) (see Appendix 11).

Percentage cover of *B. amphitrite, Polysiphonia* sp. and *E. clathrata* gradually decreased from week 0 until week 3. *X. mangle* which had an initial percentage covers of 27.88% at week 0 totally disappeared after 1 week of experiment, while *Cryptosula* sp. cover of 64.68% at week 0 also completely disappeared after 2 weeks of experiment.

Table 5.3. Weekly changes in percentage cover (%) of sessile macrofouling organisms in 20 ppt. Standard deviation (SD) in parentheses.

	Week			
	0	**1**	**2**	**3**
Plumularia sp.	52.38 (29.75)	51.74 (25.70)	52.05 (22.63)	55.03 (21.92)
Anthozoans	24.68 (26.86)	24.51 (21.25)	24.47 (24.85)	26.35 (27.04)
B. amphitrite	27.39 (19.75)	27.69 (19.75)	24.13 (30.25)	20.79 (30.25)
Polysiphonia sp.	26.82 (22.02)	31.08 (28.23)	21.68 (27.38)	20.42 (22.37)
E. clathrata	46.19 (45.67)	23.34 (23.69)	16.10 (16.47)	14.37 (14.18)
X. mangle	27.88 (3.88)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Cryptosula sp.	64.68 (64.68)	35.32 (35.32)	0.00 (0.00)	0.00 (0.00)

5.2.4. Macrofouling Community Structure in 25 ppt

Percentage cover of *Polysiphonia* sp. was significantly reduced when subjected to 25 ppt salinity. The initial cover of 59.66% at week 0 was significantly ($P < 0.05$) reduced to 3.68% cover at week 3 (Table 5.4). Percentage cover of *Plumularia* sp. fluctuated between 49.12% at week 0 to 51.59% at week 3. Covers of anthozoans were relatively constant or increased slightly to 39.08% by the 3rd week of experiment.

Table 5.4. Weekly changes in percentage cover (%) of sessile macrofouling organisms in 25 ppt. Standard deviation (SD) in parentheses.

	Week			
	0	**1**	**2**	**3**
Plumularia sp.	49.12 (23.00)	39.10 (21.83)	35.89 (29.23)	51.59 (25.95)
Anthozoans	34.76 (29.65)	8.15 (17.17)	18.01 (19.30)	39.08 (33.87)
B. amphitrite	24.03 (28.52)	27.20 (26.56)	33.40 (26.87)	15.37 (18.06)
Polysiphonia sp.	59.66 (53.35)	32.54 (30.81)	4.12 (8.36)	3.68 (7.47)*
E. clathrata	35.57 (33.00)	20.82 (22.91)	30.28 (25.81)	13.33 (18.28)
X. mangle	17.24 (3.49)	8.70 (1.81)	7.13 (2.14)	0.00 (0.00)
Cryptosula sp.	0.00 (0.00)	33.83 (43.12)	34.72 (30.09)	31.45 (26.79)

* *Indicates significant (P<0.05) difference between the initial percentage cover at week 0*

The increment rates of *B. amphitrite* were much higher in 25 ppt; its percentage cover of 24.03% at week 0 increased to 33.4% after 2 weeks of experiment. New colony of *Cryptosula* sp. (33.83% in cover) at 1st week increased to 34.72% after 2

weeks of experiment.

Cover of *E. clathrata* gradually decreased from 35.57% at week 0 to 13.33% by the week 3 while *X. mangle* gradually decreased from 17.24% cover at week 0 to 7.13% by the 2nd week and totally disappeared at the 3rd week (see Appendix 11).

5.2.5. Macrofouling Community Structure in 30 ppt

Percentage cover of anthozoans significantly ($P < 0.05$) increased from 18.88% at week 0 to 32.45% by the 3rd week while *Polysiphonia* sp. significantly ($P < 0.05$) decreased from 44.57% to 7.38% cover (Table 5.5). Percentage cover of *Plumularia* sp. gradually decreased from 51.58% at week 0 to nearly 35% cover at 2nd and 3rd week respectively. The percentage cover of *B. amphitrite* increased from week 0 until the week 3 similar to that in 25 ppt (see Appendix 11).

Covers of *E. clathrata* (37.03%) at week 0 were totally vanished by the 3rd week, while cover of *X. mangle* (7.13%) at week 0 was totally disappeared after 1 week of

Table 5.5. Weekly changes in percentage cover (%) of sessile macrofouling organisms in 30 ppt. Standard deviation (SD) in parentheses.

	Week			
	0	**1**	**2**	**3**
Plumularia sp.	51.58 (28.98)	51.30 (28.02)	33.33 (24.36)	36.35 (18.64)
Anthozoans	18.88 (24.16)	21.55 (17.90)	27.11 (27.09)	32.45 (30.85)*
B. amphitrite	21.26 (25.70)	24.13 (25.73)	27.35 (24.20)	27.25 (24.36)
Polysiphonia sp.	44.57 (33.44)	42.77 (36.55)	5.27 (14.86)	7.38 (15.15)*
E. clathrata	37.03 (40.25)	37.70 (40.98)	25.28 (18.76)	0.00 (0.00)
X. mangle	7.13 (2.14)	7.13 (2.14)	0.00 (0.00)	0.00 (0.00)
Cryptosula sp.	24.70 (27.22)	26.53 (27.04)	24.91 (23.94)	23.86 (21.79)

* *Indicates significant (P<0.05) difference between the initial percentage cover at week 0*

experiment. Percentage covers of *Cryptosula* sp. was relatively constant from week 0 until the week 3.

5.3. DISCUSSION

5.3.1. Effect of Salinity on Sessile Macrofouling Organisms

The proposed hypothesis that higher salinity was suitable for marine biofouling forms thus increases biofouling rates and conversely, low salinity decreases the biofouling rate and thus a lower biofouling biomass cannot be generalized based on the results of the present laboratory experiment. There were no clear effects of lower or higher salinity on the development or growth rates of sessile macrofouling community. However, most of the studied macrofoulers survived a wide range salinity although other factors such as natural food limitation and environment such as physical stress could cause gradual reduction in percentage cover or even mortality. The effects of salinity and their consequences on macrofouling species are discussed below.

Plumularia sp.

Plumularia sp. was not significantly affected by the range of tested salinity. However, *Plumularia* sp. survived until the 3rd week at all tested salinities suggesting its ability to tolerate a wide salinity range (i.e. 10 – 30 ppt). These results agreed with the earlier finding based on field study (Chapter 3) where its abundance was similarly high during the dry season and wet season.

According to Arndt (1984), growth rates of hydroid species were optimal at 16 ppt. However many colonial hydroids can tolerate a wide range of salinity fluctuation and become prevalent biofouling organisms in brackish estuary and freshwater habitats (e.g. Slobdkin & Bossert, 2001; Rajagopal et al., 2002; Smith et al., 2002). This further suggests that the relatively high abundance of *Plumularia* sp. during the dry and wet season in the fish farm at Jaha is attributed to its abilities to tolerate a wide range of salinity fluctuation in the estuary (see also Madin et al., 2009).

Reproduction of hydroids can occur at salinity of 10 ppt (Folino-Rorem & Indelicato, 2005); however lowering the salinity will significantly reduce their reproductive rate (Ringelband, 2001). Karbe & Dannenberg (1986) indicate that optimum salinity for rearing hydroids in laboratory was between 5 and 15 ppt. In the present study, percentage cover of *Plumularia* sp. slightly increased at salinity 10 ppt indicating that reproduction had likely occurred.

Anthozoans (unidentified sea anemones)

Anthozoans were significantly affected by lower salinity; their percentage cover was significantly reduced at salinity <15 ppt, indicating that low salinity could inhibit their growth. The present results agreed with the field study results (Chapter 3), where their abundance was significantly reduced at lower salinity (<15 ppt) during the wet season. According to Rodenbough & Ellington (1982) sea anemone can tolerate high salinity (i.e. 49 ppt) but extremely low salinity could cause detrimental effects. This further suggests that lower salinity during the wet season could be the limiting factor for the development of anemones on cage nettings (see also Madin et al., 2009).

According to Greenwood et al. (2003), osmotic stress of sea anemone does not occur at higher salinity (i.e. 30 ppt), but at lower salinity sea anemones will need to cope with hypo- and hyper-osmotic stress resulting in rapid equilibration of the coelenteron's fluid with the external medium (Rodenbough & Ellington, 1982). Thus, the relatively low development rate and thus survival rates of anemones when subjected to lower salinity (i.e. <15 ppt), could be due to osmotic stress.

Balanus amphitrite

There was no significant effect of salinity on *B. amphitrite*. Percentage cover was relatively more or less stable when subjected to the different salinities. This indicates that salinity was not an important factor determining their development in laboratory

experiments. However, there were specimens that survived until at the 3rd week, suggesting its ability to tolerate a wide salinity range although food limitation and other physical stress could inhibit development/growth rates. Food availability determines the duration of development in barnacles (Scheltema & Williams, 1982) and enough supply of food such as diatoms will increase their growth rates (Thiyagarajan et al., 2003). This further suggests that higher abundance of *B. amphitrite* during the dry season at Jaha was due to higher amount of food such as phytoplankton generated at higher salinity environment. Wong (2002) reported significantly higher ($P < 0.001$) chlorophyll-*a* in Jaha estuary during the dry season (45.82 µg 1^{-1}) than the wet season (16.72 µg 1^{-1}) during the same period of study. Thus natural foods such as phytoplankton were more readily available in the water column for barnacles during the dry season.

Polysiphonia sp.

Polysiphonia sp. was significantly influenced by salinity, the mean percentage cover significantly reduced at lower (i.e. 10 ppt) and higher (i.e. 30 ppt) salinity respectively. This suggest that extreme salinity condition could be detrimental to the development of *Polysiphonia* sp.. According to Gorham et al. (1985) and Flowers (1985) stress from high salinity will directly affect the growth of aquatic plants through insufficient turgor for cell expansion and inhibit protein production. In high salinity (i.e. >32 ppt) many macroalgae require high energy for osmotic adjustment and this negatively affects their ability to utilize dissolved inorganic for growth (Lobban & Harrison, 1997). The photosynthetic and respiratory rates of seaweed are declined in extremely high salinity (Robbins, 1978). Thus, a significant reduction in percentage cover of *Polysiphonia* sp. at 30 ppt could be due to stress from high salinity resulting in death.

Similar to the effect of high salinity, low salinity stress could cause reduction in growth, photosynthetic rate (Karsten & Kirst, 1989; Martins et al., 1999) and possibly

species disappearance (Snoeijs, 1999). This further suggests that the significant reduction in percentage cover of *Polysiphonia* sp. at 10 ppt and 15 ppt could be due to stress from low salinity which inhibit growth or even mortality. The negative implication of extreme low salinity on macroalgae is the difficulty to maintain turgor due to lost of thallus K^+ at a faster rate (Reed, 1984) and causing osmotic adjustment that concomitantly limits plant growth of species fatality (Cavalieri, 1983; Yeo, 1983).

In the present study, salinity at near 20 ppt seem to be optimal or suitable for the development of *Polysiphonia* sp.. There was relatively no significant reduction in its percentage cover. This was consistent with the finding based on field study presented in Chapter 3, where the abundance of *Polysiphonia* sp. was significantly higher during the dry season when the salinity was near 20 ppt. This further suggests that *Polysiphonia* sp. is an estuarine or euryhaline species which survives medium salinity condition or moderate salinity fluctuation.

Relatively low light intensity in the laboratory could reduce the growth rates and thus the survival of *Polysiphonia* sp.. According to Demetropoulos & Langdon (2004) growth rates of many Rhodophyta as well as other aquatic plant will increase at high light intensity and temperature. There was no measurement of light intensity in the laboratory although it was obviously lower than those observed in the fish farm. In addition, low amount of essential nutrient could concomitantly cause stress that inhibits the reproduction of *Polysiphonia* sp..

Enteromorpha clathrata

There was no significant effect of salinity on *E. clathrata*, its percentage cover was similarly reduced whether subjected to higher or lower salinity treatments. This suggests that the tested salinity levels do not affect its development. *E. clathrata* survived for 2 – 3 weeks, further suggesting its ability to tolerate a wide range of salinity. According to

Moll & Deikman (1995), *Enteromorpha* can tolerate elevated salinity (i.e.15 to 31 ppt). However, with sufficient nutrient such as phosphorus and nitrogen, *Enteromorpha* can grow at faster rates (e.g. Fujita et al., 1988; Duke et al., 1989; Gordon & Mc Comb, 1989; Jorgensen et al., 2002).

The extreme level of salinity either at hypo or hypersaline conditions can cause physiological stress on *Enteromorpha* (Martins et al., 1999; Pringle, 1986; Young et al., 1987a; 1987b; Kirst, 1989; Martins et al., 1999). However, according to Martins et al. (1999) the effects of salinity on *Enteromorpha* were more prominent at lower salinity than at higher salinity. Lower salinity could reduce pigmentation, biomass (Sfriso et al., 1987), suppressed reproduction (Pringle, 1986) and the ability to regulate internal solutes that would cause death within 6 days (Edwards et al., 1988; Young et al., 1987a; Martins et al., 1999). Thus, the lower abundance of *E. clathrata* during the wet season in a fish farms at Jaha could be due to lower salinity stress and probably competition for nutrients with the more dominant *Polysiphonia* sp..

However, low light intensity in the laboratory could have responsibly reduced the photosynthetic rate and caused stress result to *E. clathrata*, as similar to *Polysiphonia* sp. According to Hillman et al. (1995) poor light intensity can cause severe decline in the growth rate of aquatic plants.

Xenostrobus mangle

There was no clear effect of higher or lower salinity treatments on the percentage cover of *X. mangle*, suggesting that salinity may have little effect on its development. The experimental results contradict the findings from the field study (Chapter 3), where the abundance of *X. mangle* was significantly higher during the wet season (i.e. < 20 ppt) than during the dry season (higher salinity).

Most of the *X. mangle* however died within the first two weeks of the experiment

indicating a low survival rate. Their survival rates were relatively lower than the other macrofoulers suggest that limited amount of natural food such as phytoplankton while the artificial feed may not be suitable for *X. mangle.*

According to Phillips (1977) and Struck et al. (1997) the uptake rates of several food element required by mussels are increased at lower salinity. This further suggests that relatively higher abundance of *X. mangle* in the fish farm at Jaha during the wet season is attributed to its ability to obtained food under the low salinity condition. In the laboratory experiment, other factors that may influence the survival rates and thus the growth of *X. mangle* such as the fluctuation in pH. Low pH has been shown to be toxic to bivalve molluscs (Bamber, 1987; 1999). Although the pH was properly maintained during the experiment, fluctuation continued to occur due to feeding and accumulation of waste material.

Cryptosula sp.

Cryptosula sp. was not significantly ($P > 0.05$) influenced by salinity, there was no significant increment or reduction of its percentage cover when subjected to higher (i.e. >20 ppt) or lower (i.e. <15 ppt) salinity treatments. This suggests that the range of salinity does not affect its development. The present result does not support the field study (Chapter 3), where *Cryptosula* sp. was thought to be dominant in the fish farm at Sangga Besar due to its higher salinity environment. Hence, other factors such as competition for limited attachment surface especially from the more dominant *Plumularia* sp., and the relatively stronger water flow as well as less polluted fish farm at Jaha may contribute to low abundance of *Cryptosula* sp. during the experiment.

Cryptosula sp. survived until for 2 or 3 weeks when subjected to different salinities, suggesting its capability to tolerate a wide range of salinity although the low amount of natural food or unsuitable given feed could inhibit their further development similar to

the other macrofoulers. There were new colonies after one week of culturing at 15 ppt and 25 ppt respectively. A number of authors indicate that many bryozoans species could tolerate a wide range salinity fluctuation from oligohaline (3 %) to hyperhaline conditions (i.e. 42 ppt), and are capable of colonizing habitats where life is usually impossible for many other organisms (e.g. Winston, 1977; Occhipinti, 1981 cited in Badve & Sonar, 1995; Poluzzi & Agnoletto, 1988; Gordon & Mawatari, 1992; Occhipinti & d'Hondt, 1981; Freitas et al., 1994).

Low night temperature could also give stress to the *Cryptosula* sp. causing lower growth rates at high salinities. According to Smith et al. (1998) variations in water temperature influence the growth rate of bryozoans.

5.3.2. Effects of Other Factors

In the present study, stress from other factors such as food limitation and unsuitable food rather than salinity could influence the development and thus the survival rates of sessile macrofoulers when subjected to laboratory experiments. This was possible since most of the studied macrofouling organisms survived for 3 weeks or at least 2 weeks when subjected to different salinities. In the fish farm, variability in the abundance of macrofoulers during the dry and wet seasons could be due to different amounts of food i.e. phytoplankton availability between seasons (see Wong, 2002; Madin et al., 2009).

According to Santschi (1995) the regeneration of phosphorus in estuarine environment is maximum at high salinity while many important suspended particulates increased with increasing salinity (Zwolsman & van Eck, 1999). These further suggest that higher abundance of particularly the algal fouling in a fish farm during the dry season could be due to higher amount of nutrient regenerated at high salinity environment, while low salinity during the wet season generates lesser amount of nutrient resulting in less algal fouling. Davies & Eyre (2005) found that total nitrogen in

estuary will be removed at very low salinity, while concentration of silicate and phosphate stayed more or less constant below salinities of 10 – 15 ‰ (Froelich, 1988).

Spatial distributions of phytoplankton are also influenced by salinity. Phytoplankton such as diatoms grow rapidly at high salinity i.e. 34 ppt (e.g. Opute, 1990; Kononen et al., 1996; 1999) and peak abundance will occur at higher temperature (Kim et al., 2004). This further suggests that high salinity during the dry season could generate more phytoplanktonic food for invertebrate macrofoulers.

In the laboratory experiment, types of food necessary for sessile macrofoulers such phytoplankton and nutrients were not well considered. It could be possible that foods given (i.e. pellet feed, ground trash fish and commercial coral feed) to the macrofoulers did not provide ample nutrition to support their development.

5.4. CONCLUSION

Based on the laboratory experiment, the proposed hypothesis that higher salinity could increase the biofouling rates or reduce their rates at lower salinity cannot be generalized. The study shows that different salinities whether at higher or at lower salinity does not necessarily increase or reduce the rates of macrofouling. The study suggests that salinity alone would not determine the community structure of macrofouling assemblages but rather in combination with other factors such as food availability, larval availability, competition, essential nutrients and other environment and physical factors. Most of the studied macrofoulers survives at least for a week or until for 3 weeks, indicating their ability to tolerate a wide range of salinity and therefore they are prevalent foulers of floating net-cages in estuarine waters.

CHAPTER 6

NUTRIENT AND CHLOROPHYLL-*a* CONCENTRATION OF FISH CULTURE WATER IN RELATION TO BIOFOULING DEVELOPMENT

Summary of Important Finding

There was no significant ($P > 0.05$) increase in dissolved inorganic nitrogen (NH_3-N, NO_2-N and NO_3-N) concentration immediately after fish feeding whether with trash fish or pellet feed, however PO_4^{-3} increased significantly ($P < 0.05$) after feeding (i.e. at 30^{th} minute) with pellet feed (P) suggesting that the feed pellets had or released more phosphate than trash fish (T). The overall mean concentration of nutrients (i.e. NH_3-N, NO_2-N, NO_3-N and PO_4^{-3}) within 120 minutes of measurement was usually higher inside net-cages than outside it (O), suggesting that fish rearing whether given trash fish or pellet feed, contributed to nutrient enrichment of the culture water. However, the chlorophyll-*a* concentration was not consistent with the level of nutrient concentration indicating no clear effect of nutrient enrichment, including its effect on biofouling.

6.1. INTRODUCTION

Nutrients play an important role in productivity and water quality of marine and estuarine environments because of their role in the functioning of biological systems. The term nutrient refers to anything beside water and carbon dioxide (CO_2) that is vital for plant in the synthesis of organic matter or skeletal material (Stowe, 1987). Major nutrients are nitrogen and phosphorus which are in the form of dissolved inorganic or organic compounds. Dissolved inorganic nitrogen comprises mainly of dissolved nitrogen gas (N_2), ammonia (NH_4), nitrate (NO_3-N) and nitrite (NO_2-N), while dissolved inorganic phosphorus comprises of PO_4 ions. Organic compounds include those bound up in plankton or biodetritus (Haris, 1986). Other important nutrients present as a mineral include iron (Fe) and silicate (Si), which have significant effects on living

marine organisms as well as primary productivity (e.g. Martin, 1994; Martin et al., 1995; Coale, 1996; Boyd, 2000; Gobler et al., 2002).

Similar to non-fouling organisms, biofoulers themselves rely on nutrient to sustain life. Based on the present findings as reported in Chapter 3, the relative abundance of several macrofoulers and thus the total wet biomass of net panels placed inside the net-cages (i.e. with fish rearing and fish feed input) were significantly higher than outside the net-cages (i.e. without fish rearing and fish feed input). Among the suggested reasons for this was the availability of increased nutrient as well as food or organic matter from fish rearing activities including uneaten fish feed particulates and fish faeces. The nutrients are expected to promote phytoplankton blooms inside the net-cages (see also Madin et al., 2009).

Several authors are of the opinion that nutrient enrichment and increased food availability contribute to higher biofouling in fish culture farms. For example, Ruokolahti (1988) suggested that species composition, abundance and development rates of macrofouling organisms in fish culture farm reflect in some way the added nutrients and organic loading associated with its operation. Qian et al. (2001) noted that fish culture farms are heavily fouled with filter feeders, because the nutrient enriched environment provides increased food supply. Coasta-Pierce & Bridger (2002) indicates that increase of fouling level in aquaculture can be expected owing to the increased level of nutrients. Furthermore, Dubost et al. (1996) noted that the development of freshwater biofoulers can be modified by the nutrient content of fish farm water.

Relatively higher development rates and thus abundance of several macrofoulers inside the net-cages as compared to outside it have been discussed in Chapter 3. In particular, algal fouling such as by *Polysiphonia* sp. and *E. clathrata* are known to be

dependent on nutrient such as phosphorus and nitrogen to grow rapidly (e.g. Jorgensen et al., 2002). Fong et al. (1993a; 1994a; 1994b) show that a high nutrient pulse greatly enriched with nitrogen favors *Enteromorpha* growth, while limitation of particularly nitrogen will limit its growth and biomass.

The direct effects of nutrient enrichment on invertebrate fouling such as *B. amphitrite*, anthozoans and *X. mangle* are not understood. However, nutrient enrichment encourages phytoplankton blooms which in turn serve as food for these organisms. The growth limitation of phytoplankton as primary producer is correlated to the uptake of nutrient, whereas enhancement of nitrogen or phosphorus can increase their biomass and productivity under sufficient light condition (e.g. Howarth, 1988; Beukema, 1991; Nixon, 1995; Posey et al., 1995; Becker, 1996; Pitta et al., 1998; Downing et al., 1999; Posey et al., 2002). Nutrient generated from fish culture farm has a redfield ratio of N:P close to 7:1 w/w (Aure & Stigebrandt, 1990), potentially a well-balanced nutrient mix for phytoplankton growth (Wu, 1995). However, the relationships between nutrient enrichment, phytoplankton blooms and thus macrofoulers development in floating net-cages have not been assessed. Thus, one purpose of this study was to determine the phytoplankton standing stock based on chlorophyll-*a* concentration so as to determine its relationship with nutrient enrichment and thus with macrofouler development.

The relatively higher abundance of non-sessile macrofoulers inside the net-cages as compared to outside it (Chapter 3) suggests that nutrient and organic enrichment from fish rearing activities stimulate a wide range of feeding strategies in non-sessile organisms. A number of studies have indicated that nutrient and organic enrichment as well as primary production contribute to the variation in the population and biomass patterns of several estuarine fauna (e.g. Tsutsumi et al., 1990; Wolfrath, 1992; Posey et al., 1995; Heip et al., 1995).

The main contributor of nutrient and organic enrichment inside the floating net-cages has not been assessed, although feed wastage (i.e. uneaten feed particulate) and fish faeces including metabolite waste of cultivated fish are likely to contribute to nutrient enrichment of culture water and thus the growth of macrofouling assemblages on cage nettings (see Chapter 4). The extent of the effects of fish feed wastage, fish faeces and metabolite waste on nutrient and organic enrichment of culture water are difficult to conclude. However, several authors suggested that feed wastage and other waste material especially from low quality fish feed such as trash fish is an important source that contributes to the nutrient and organic enrichment in fish culture farms (e.g. Ackefors & Enell, 1990; Seymour & Bergheim, 1991; Qian et al., 2001; Coasta-Pierce & Bridger, 2002). Thus, another purpose of this study was to determine the leached dissolved nutrient concentration of culture water as possibly generated from feed wastage as well as fish faeces and metabolite waste of cultured fish in floating net-cages. Two types of fish feed were used; trash fish the normal feed used for fish culture in MMFR which is considered to give high feed wastage, and pellet feed which is considered to give less feed wastage.

6.2. RESULTS

6.2.1. Environmental Parameters

In all tidal phases, pH readings did not significantly ($P > 0.05$) change immediately after fish feeding i.e. at time 0, whether with pellet or trash fish feed (Figure 6.1a). The overall mean pH after 120 minutes (from fish feeding) was not significantly ($P > 0.05$) different among treatments (pellet, trash fish and outside net-cage) during the flood, slack and ebb water respectively.

Temperature did not significantly ($P > 0.05$) change immediately after fish feeding whether with pellet or trash fish feed at all tidal phases (Figure 6.1b). During the

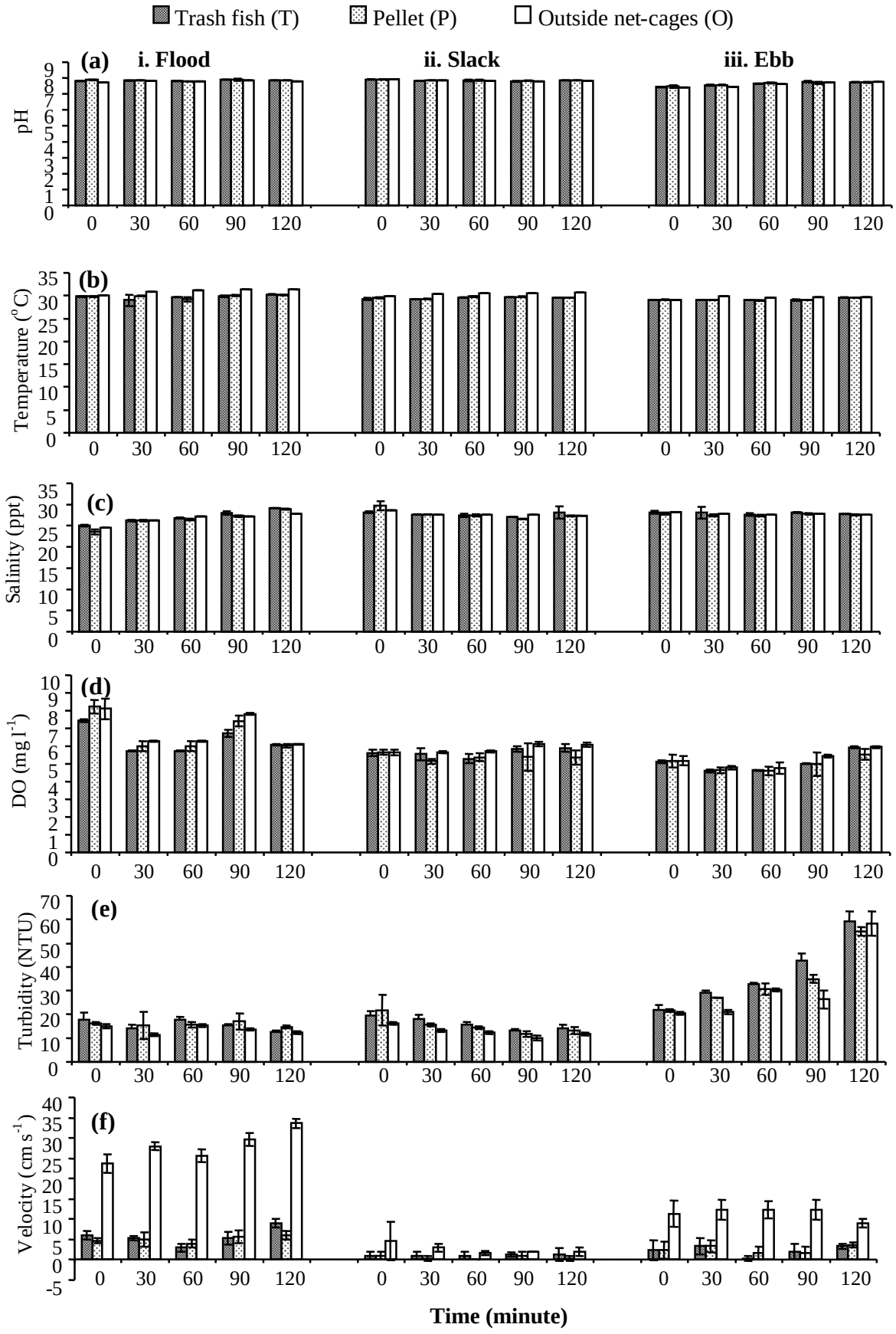


Figure 6.1. Mean (± SD) pH, temperature, salinity, DO, turbidity and current velocity recorded before feeding at minute 0 and at 30, 60, 90 and 120 minutes after feeding in trash fish cages (T), pellet cages (P) and outside net-cages (O), during the flood (i), slack (ii) and ebb (iii) water in a fish farm at Jaha.

flood and slack water, mean temperature at 120 minutes was statistically ($P < 0.05$) higher outside the net-cages than in pellet or trash fish feed cages. However, during the ebb water, temperature was not significantly ($P > 0.05$) different among treatments.

Salinity did not significantly ($P > 0.05$) change immediately after fish feeding in all tidal phases (Figure 6.1c). During the flood water, mean salinity after 120 minutes was statistically ($P < 0.05$) higher in trash fish cages than in pellet feed cages or outside the net-cages. However, during the slack and ebb water there were no significant ($P > 0.05$) different in mean salinity among treatments.

There were no significant ($P > 0.05$) changes in dissolved oxygen (DO) reading immediately after fish feeding whether with pellet or trash fish feed during the flood, slack and ebb water (Figure 6.1d). During the flood and slack water, mean DO after 120 minutes was statistically ($P < 0.05$) higher outside the net-cages than in pellet or trash fish cages. However, there were no significant ($P > 0.05$) differences among treatments during the ebb water.

Turbidity was statistically ($P < 0.05$) different 90 minutes or more after fish feeding during ebb water (Figure 6.1e). However, there were no significant ($P > 0.05$) changes during the flood and slack water. In all tidal phases, mean turbidity reading at 120 minute was statistically ($P < 0.05$) higher in trash fish or pellet feed cages than outside the net-cage.

In all tidal phases, current velocity was not significantly ($P > 0.05$) different immediately after fish feeding (Figure 6.1f). Mean current velocity over 120 minutes was statistically ($P < 0.05$) higher outside the net-cages than inside it (i.e. pellet or trash fish cages) at all tidal phases.

6.2.2. Nutrient Concentrations

6.2.2.1. Ammonia-Nitrogen (NH_3-N)

There was no significant ($P > 0.05$) increase in ammonium concentration immediately after fish feeding whether with trash fish or pellet feed during the flood water (Figure 6.2i). The overall mean concentration at 120 minutes after fish feeding was significantly ($P < 0.05$) higher in trash fish feed cages (3.52 µmol l^{-1}) than in pellet cages (2.88 µmol l^{-1}) and outside the net-cages (2.33 µmol l^{-1}).

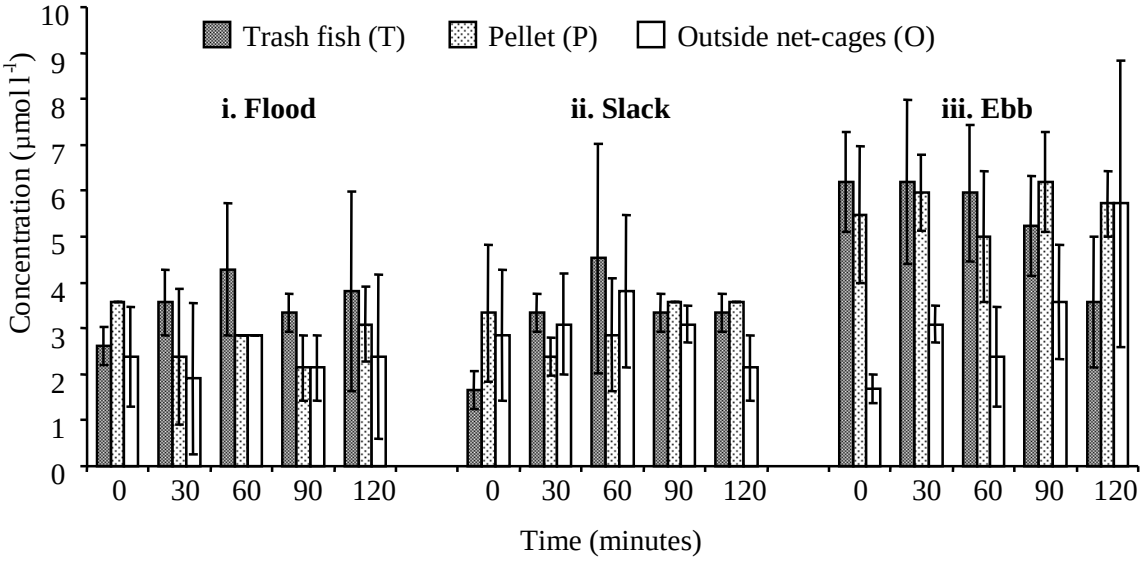


Figure 6.2. Mean (± SD) concentration (µmol l^{-1}) of Ammonia-Nitrogen (NH_3-H) recorded before feeding at time 0 and at 30, 60, 90 and 120 minutes after feeding in trash fish cages (T), pellet cages (P) and outside net-cages (O), during the flood (i), slack (ii) and ebb (iii) water in a fish farm at Jaha.

Similar to the result of flood water, there were also no significant ($P > 0.05$) increase in ammonium concentrations immediately after feeding whether with pellet or trash fish feed during slack water (Figure 6.2ii). However, unlike the result of flood water, there were no significant ($P > 0.05$) differences among mean concentrations of ammonium at 120 minutes after feeding; these were 3.23 µmol l^{-1}, 3.14 µmol l^{-1} and 3.00 µmol l^{-1} for trash fish cages, pellet cages and outside the net-cages respectively.

There was also no significant ($P < 0.05$) increase in ammonium concentration immediately after fish feeding, whether with pellet or trash fish feed during ebb water (Figure 6.2iii). However, mean concentration at 120 minutes after feeding was significantly ($P < 0.05$) higher in pellet (5.66 µmol l^{-1}) or trash fish (5.43 µmol l^{-1}) cages than outside the net-cages (3.19 µmol l^{-1}) (detailed data appended in Appendix 12A).

6.2.2.2. Nitrite-Nitrogen (NO_2-N)

There was no significant ($P > 0.05$) increase in nitrite concentration immediately after fish feeding whether with trash fish or pellet feed during the flood water. The concentration was initially high in both feeding treatments but reduced thereafter. Concentration decreased significantly ($P < 0.05$) from 1.04 µmol l^{-1} at 0^{th} minute to 0.33 µmol l^{-1} at 30^{th} minute in trash fish feed cages, while it decreased from 0.93 µmol l^{-1} to 0.29 µmol l^{-1} in pellet feed cages (Figure 6.3i). There were no significant ($P > 0.05$) differences in mean concentration of nitrite among treatments after 120 minutes. The mean were 0.48 µmol l^{-1}, 0.45 µmol l^{-1} and 0.39 µmol l^{-1} for trash fish feed cages, pellet cages and outside the net-cages respectively.

Similar to the results of flood water, there was also no significant ($P > 0.05$) increase in nitrite concentration immediately after fish feeding during slack water (Figure 6.3ii). However, mean concentrations after 120 minutes were significantly ($P <0.05$) higher in pellet (0.31 µmol l^{-1}) and trash fish (0.31 µmol l^{-1}) feed cages as compared to outside the net-cages (0.22 µmol l^{-1}).

There was also no significant ($P > 0.05$) increase in nitrite concentration immediately after fish feeding whether with pellet or trash fish feed during the ebb water (Figure 6.3iii). Mean concentration after 120 minutes of measurement was not significantly ($P > 0.05$) different among treatments. These were 0.33 µmol l^{-1}, 0.31µmol l^{-1} and 0.28 µmol l^{-1} for trash fish feed cages, pellet feed cages and outside the net-cages

respectively (see Appendix 12B).

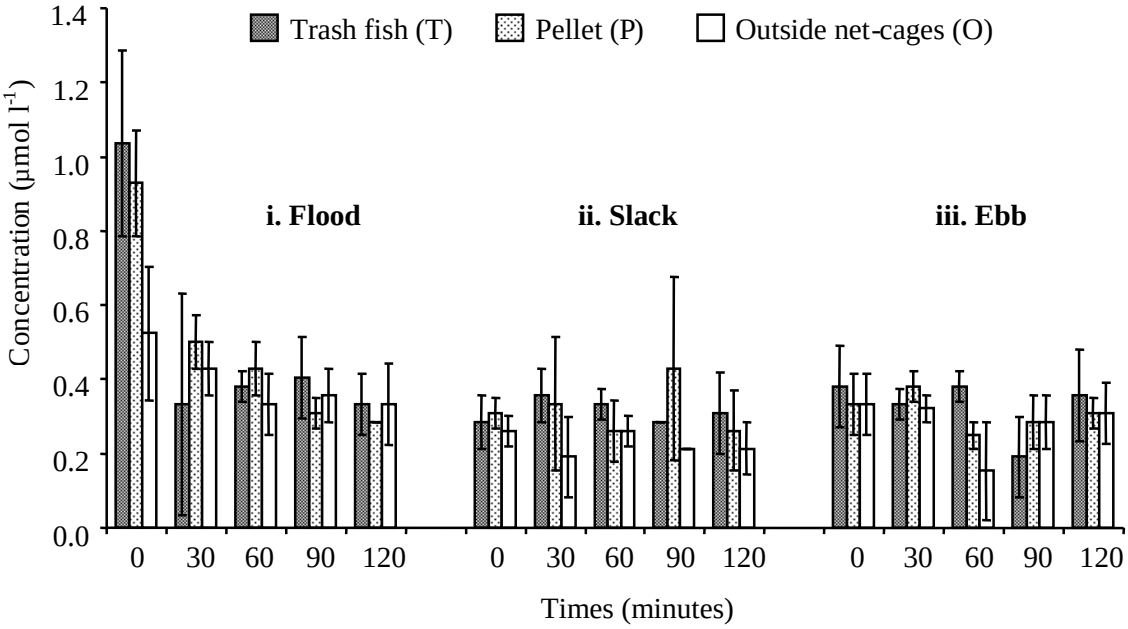


Figure 6.3. Mean (± SD) concentration (µmol l^{-1}) of Nitrite-Nitrogen (NO_2-N) recorded before feeding at minute 0 and at 30, 60, 90 and 120 minute after feeding in trash fish cages (T), pellet cages (P) and outside net-cages (O), during the flood (i), slack (ii) and ebb (iii) water in a fish farm at Jaha.

6.2.2.3. Nitrate-Nitrogen (NO_3-N)

There was no significant increase in nitrate concentration immediately after fish feeding whether with trash fish feed or pellet feed during the flood water (Figure 6.4i). The overall mean concentrations of nitrate after 120 minutes were not significantly (P > 0.05) different among treatments; these were 2.76 µmol l^{-1}, 2.73 µmol l^{-1} and 2.76 µmol l^{-1} for trash fish cages, pellet cages and outside the net-cages respectively.

Similar to results of the flood water, nitrate concentration did not increase significantly (P > 0.05) after fish feeding during the slack water (Figure 6.4ii). However, the overall mean concentration after 120 minutes was significantly (P < 0.05) higher in trash fish feed cages with 1.95 µmol l^{-1} as compared to 1.52 µmol l^{-1} and 1.57 µmol l^{-1} for pellet feed cages and outside the net-cages respectively.

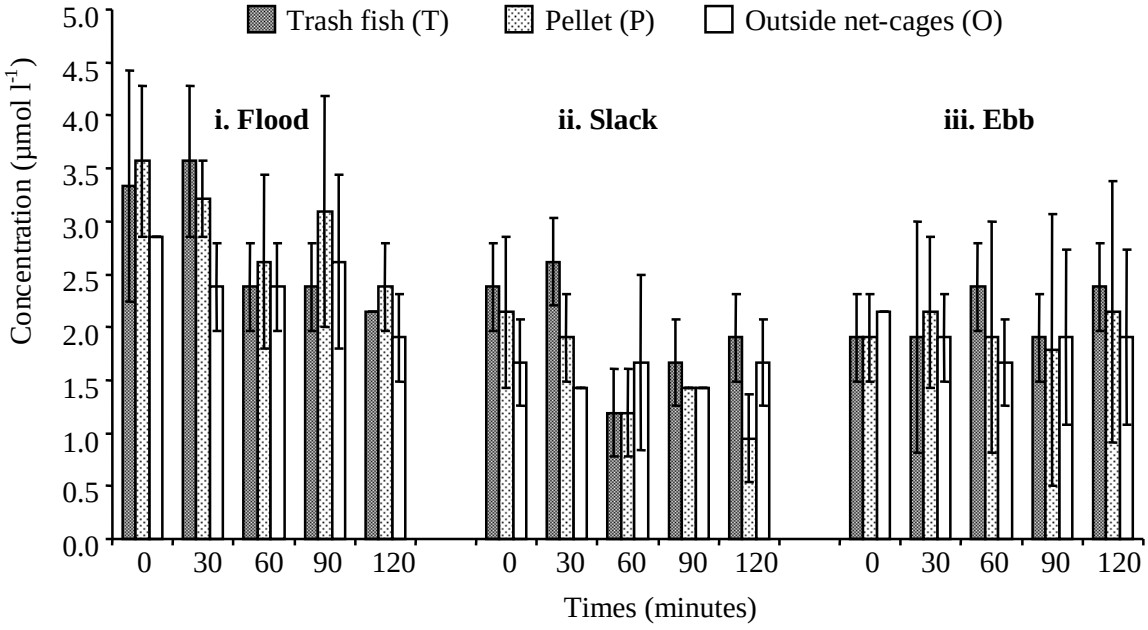


Figure 6.4. Mean (± SD) concentration (µmol l^{-1}) of Nitrate-Nitrogen (NO_3-N) recorded before feeding at minute 0 and at 30, 60, 90 and 120 minutes after feeding in trash fish cages (T), pellet cages (P) and outside net-cages (O), during the flood (i), slack (ii) and ebb (iii) water in a fish farm at Jaha.

There were also no significant (P > 0.05) increase in nitrate concentration immediately after fish feeding whether with trash fish or pellet feed during the ebb water (Figure 6.4iii). Mean concentrations after 120 minutes were not significantly (P > 0.05) different among treatments. These were 2.09 µmol l^{-1}, 1.97 µmol l^{-1} and 1.90 µmol l^{-1} respectively for trash fish feed cages, pellet feed cages and outside the net-cages (see Appendix 12C).

6.2.2.4. Reactive Phosphate (PO_4^{-3})

There was no significant (P > 0.05) increase in concentration of phosphate immediately after fish feeding whether with pellet or trash fish feed during the flood water (Figure 6.5i). Overall mean concentrations after 120 minutes were not significantly (P > 0.05) different among the treatments. It was higher in pellet feed cages (3.64 µmol l^{-1}) than outside the net-cages (2.42 µmol l^{-1}) or trash fish cages (1.29 µmol l^{-1}) although the difference was insignificant (see Appendix 12D).

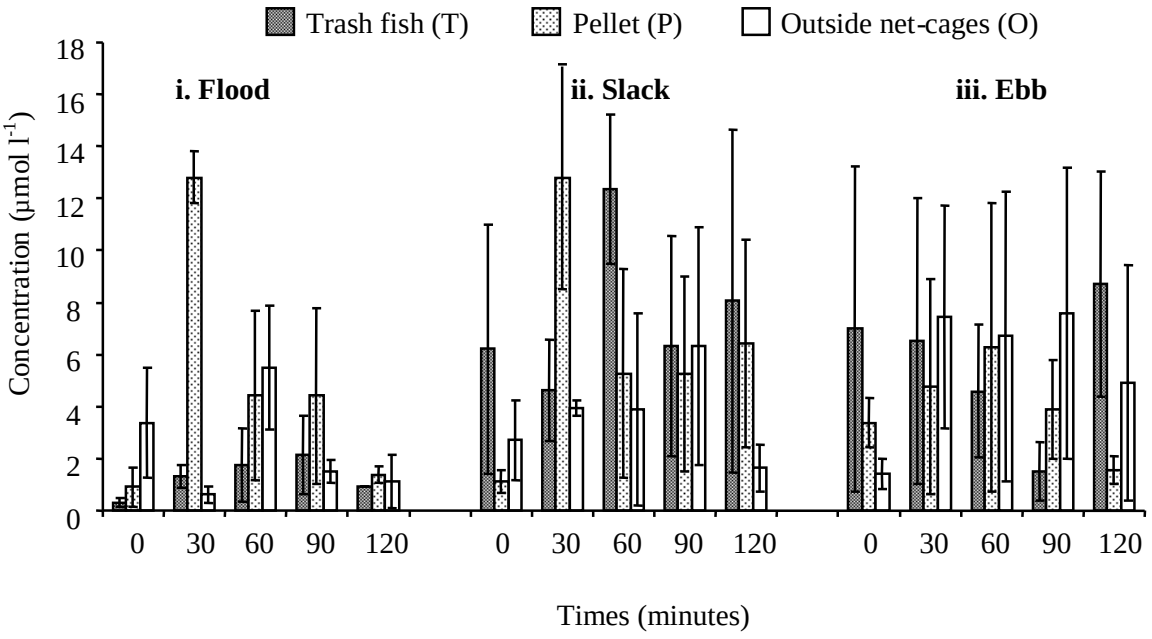


Figure 6.5. Mean (± SD) concentration (µmol l^{-1}) of Reactive Phosphate (PO_4^{-3}) recorded before feeding at minute 0 and at 30, 60, 90 and 120 minutes after feeding in trash fish cages (T), pellet cages (P) and outside net-cages (O), during the flood (i), slack (ii) and ebb (iii) water in a fish farm at Jaha.

During the slack water, concentration of phosphate after fish feeding with pellet feed significantly ($P < 0.05$) increased from 1.12 µmol l^{-1} before feeding (minute 0) to a highest of 12.81 µmol l^{-1} at 30th minute (Figure 6.5ii). However, there were no significant ($P > 0.05$) increase after feeding with trash fish feed, concentration increased from 6.21 µmol l^{-1} before feeding (minute 0) to a highest of 12.35 µmol l^{-1} at the 60th minute. The overall mean concentrations of phosphate after 120 minutes were significantly ($P < 0.05$) higher in trash fish feed (7.51 µmol l^{-1}) and pellet feed (6.17 µmol l^{-1}) cages than outside the net-cages (4.01 µmol l^{-1}).

During ebb water, concentration of reactive phosphate increased immediately after fish feeding whether with pellet or trash fish feed, although the increase was insignificant ($P > 0.05$) (Figure 6.5iii). Mean concentrations after 120 minutes were not significantly ($P > 0.05$) different among treatments. These were 5.66 µmol l^{-1}, 3.81

µmol l^{-1} and 5.66 µmol l^{-1} in trash fish cages, pellet cages and outside the net-cages, respectively.

6.2.3. Chlorophyll-*a*

During the flood water, chlorophyll-*a* concentrations were consistently high in pellet and trash fish feed cage than outside net-cages. The highest concentration of 48.21 µg l^{-1} was obtained at the 30th minute for pellet feed cage while 48.41 µg l^{-1} was obtained at the 120th minute in trash fish feed cages. Outside the net-cages, a highest concentration of 35.41 µg l^{-1} was obtained at start of experiment (i.e. 0 min.) (Figure 6.6i). Mean concentration of chlorophyll-*a* after 120 minutes was significantly ($P < 0.05$) higher for pellet feed (41.42 µg l^{-1}) and trash fish feed (37.78 µg l^{-1}) cages than outside the net-cages (23.46 µg l^{-1}) (see Appendix 12E).

During slack water, concentration of chlorophyll-*a* was inconsistent or relatively reduced in all treatments. The highest concentrations of 93.41 µg l^{-1} was obtained at the 30th minute for pellet feed cages, while a highest of 90.74 µg l^{-1} was obtained at minute 0 for trash fish feed cages. Concentration fluctuated between 48.60 µg l^{-1} and 85.02 µg l^{-1} outside the net-cages (Table 6.6ii). Mean concentration of chlorophyll-*a* after 120 minutes of measurements was not significantly ($P > 0.05$) different among treatments. It was 86.96 µg l^{-1}, 79.94 µg l^{-1} and 71.01 µg l^{-1} for pellet feed cages, trash fish feed cages and outside the net-cages respectively.

During ebb water, chlorophyll-*a* concentrations in pellet and trash fish cages were relatively increased. The highest concentration of 46.24 µg l^{-1} was obtained at 90th minute for pellet feed cages, while the highest concentration of 37.50 µg l^{-1} for trash fish feed also obtained at 90th minute (Figure 6.6iii). Concentration was lower outside net-cages, the highest of 22.48 µg l^{-1} was obtained at 30th minute. The mean concentration of chlorophyll-*a* after 120 minutes was significantly ($P < 0.05$) higher in

pellet feed (34.54 µg l^{-1}) and trash fish feed (28.39 µg l^{-1}) cages than outside the net-cages (21.56 µg l^{-1}).

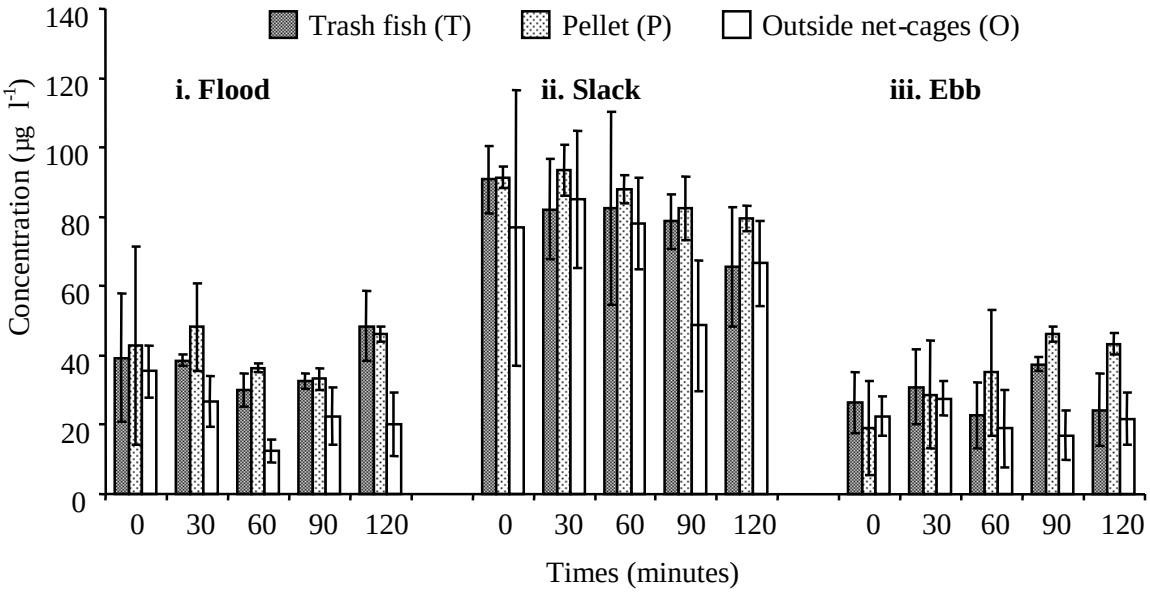


Figure 6.6. Mean (± SD) concentration (µg l^{-1}) of chlorophyll-*a* recorded before feeding at minute 0 and at 30, 60, 90 and 120 minutes after feeding in trash fish cages (T), pellet cages (P) and outside net-cages (O), during the flood (i), slack (ii) and ebb (iii) water in a fish farm at Jaha.

6.2.4. Correlation Analysis

During the flood water, nitrite concentration in trash fish feed and outside the net-cages was significantly ($P < 0.05$) correlated (r = 0.66) with chlorophyll-*a* (Table 6.1). However, this was not observed in pellet cages. There were no significant ($P < 0.05$) correlation between ammonium, nitrate and phosphate with chlorophyll-*a* in all treatments.

During the slack water, concentrations of nitrite and nitrate in trash fish and pellet cages were significantly ($P < 0.05$) correlated with chlorophyll-*a* (r = -0.55; r = -0.58) (Table 6.2). However, there were no significant ($P > 0.05$) correlation between nutrients and chlorophyll-*a* outside the net-cages.

During the ebb water, nitrate concentration in trash fish cages was significantly ($P <$

Table 6.1. Summary table of correlation matrix among nutrients (NH_3-N, NO_2-N, NO_3-N, PO_4^{-3}) and chlorophyll-*a* inside trash fish cages (a), pellet cages (b) and outside the net-cages (c) during the flood water.

(a) Trash fish cages (T)

	NH_3-N	NO_2-N	NO_3-N	PO_4^{-3}	Chl-*a*
NH_3-N	1.00	0.41	**0.60***	-0.09	0.10
NO_2-N	0.41	1.00	0.32	-0.27	**0.66***
NO_3-N	**0.60***	0.32	1.00	0.19	-0.13
PO_4^{-3}	-0.09	-0.27	0.19	1.00	-0.36
Chl-*a*	0.10	**0.66***	-0.13	-0.36	1.00

(b) Pellet cages (P)

	NH_3-N	NO_2-N	NO_3-N	PO_4^{-3}	Chl-*a*
NH_3-N	1.00	-0.43	-0.27	0.04	0.02
NO_2-N	-0.43	1.00	0.39	-0.50	-0.12
NO_3-N	-0.27	0.39	1.00	-0.21	-0.17
PO_4^{-3}	0.04	-0.50	-0.21	1.00	-0.34
Chl-*a*	0.02	-0.12	-0.17	-0.34	1.00

(c) Outside net-cage (O)

	NH_3-N	NO_2-N	NO_3-N	PO_4^{-3}	Chl-*a*
NH_3-N	1.00	-0.03	-0.17	0.05	**-0.53***
NO_2-N	-0.03	1.00	0.02	0.00	**0.57***
NO_3-N	-0.17	0.02	1.00	0.05	0.33
PO_4^{-3}	0.05	0.00	0.05	1.00	0.04
Chl-*a*	**-0.53***	**0.57***	0.33	0.04	1.00

** Indicates significant correlation for factor ($P < 0.05$)*

Table 6.2. Summary table of correlation matrix among nutrients (NH_3-N, NO_2-N, NO_3-N, PO_4^{-3}) and chlorophyll-*a* inside trash fish cages (a), pellet cages (b) and outside the net-cages (c) during the slack water.

(a) Trash Fish (T)

	NH_3-N	NO_2-N	NO_3-N	PO_4^{-3}	Chl-*a*
NH_3-N	1.00	0.25	-0.45	0.22	-0.03
NO_2-N	0.25	1.00	0.02	0.20	**-0.55***
NO_3-N	-0.45	0.02	1.00	-0.25	0.16
PO_4^{-3}	0.22	0.20	-0.25	1.00	-0.50
Chl-*a*	-0.03	**-0.55***	0.16	-0.50	1.00

(b) Pellet (P)

	NH_3-N	NO_2-N	NO_3-N	PO_4^{-3}	Chl-*a*
NH_3-N	1.00	0.23	-0.30	-0.50	0.03
NO_2-N	0.23	1.00	0.51	-0.02	**-0.58***
NO_3-N	-0.30	0.51	1.00	-0.28	**-0.58***
PO_4^{-3}	-0.50	-0.02	-0.28	1.00	0.04
Chl-*a*	0.03	**-0.58***	**-0.58***	0.04	1.00

(c) Outside the net-cages (O)

	NH_3-N	NO_2-N	NO_3-N	PO_4^{-3}	Chl-*a*
NH_3-N	1.00	0.11	-0.02	0.27	-0.23
NO_2-N	0.11	1.00	-0.04	0.00	0.44
NO_3-N	-0.02	-0.04	1.00	0.00	0.19
PO_4^{-3}	0.27	0.00	0.00	1.00	-0.28
Chl-*a*	-0.23	0.44	0.19	-0.28	1.00

** Indicates significant correlation for factor ($P < 0.05$)*

0.05) correlated (r = -0.56) with chlorophyll-*a* (Table 6.3). There were no significant (*P* > 0.05) correlations between nutrients and chlorophyll-*a* in pellet given cage or outside the net-cages.

Table 6.3. Summary table of correlation matrix among nutrients (NH_3-N, NO_2-N, NO_3-N, PO_4^{-3}) and chlorophyll-*a* inside trash fish cages (a), pellet cages (b) and outside the net-cages (c) during the ebb water.

(a) Trash Fish (T)					
	NH_3-N	NO_2-N	NO_3-N	PO_4^{-3}	Chl-*a*
NH_3-N	1.00	0.12	-0.26	-0.27	-0.24
NO_2-N	0.12	1.00	0.35	-0.11	0.28
NO_3-N	-0.26	0.35	1.00	-0.05	**0.56***
PO_4^{-3}	-0.27	-0.11	-0.05	1.00	0.12
Chl-*a*	-0.24	0.28	**0.56***	0.12	1.00
(b) Pellet (P)					
	NH_3-N	NO_2-N	NO_3-N	PO_4^{-3}	Chl-*a*
NH_3-N	1.00	0.07	**-0.58***	0.16	0.12
NO_2-N	0.07	1.00	0.14	-0.24	-0.14
NO_3-N	**-0.58***	0.14	1.00	-0.36	-0.04
PO_4^{-3}	0.16	-0.24	-0.36	1.00	0.14
Chl-*a*	0.12	-0.14	-0.04	0.14	1.00
(c) Outside net-cages (O)					
	NH_3-N	NO_2-N	NO_3-N	PO_4^{-3}	Chl-*a*
NH_3-N	1.00	0.29	-0.05	**0.57***	0.13
NO_2-N	0.29	1.00	0.06	0.06	0.22
NO_3-N	-0.05	0.06	1.00	-0.13	-0.29
PO_4^{-3}	**0.57***	0.06	-0.13	1.00	-0.09
Chl-*a*	0.13	0.22	-0.29	-0.09	1.00

** Indicates significant correlation for factor (P < 0.05)*

6.3. DISCUSSION

In the present study, dissolved ammonium (NH_3-N) contributed about 60% of the inorganic nitrogen nutrient in the fish farm at Jaha. During the flood and slack water, leaching rates of ammonium during the first 60 minutes after feeding was relatively higher in trash fish cages than in pellet cages indicating that the former released ammonium at a faster rate probably due to the use of partly rotten fishes. In addition, food given to the cultured fishes could stimulate excretion leading to higher ammonium in the water column. Ammonium has been recognized to be the major catabolic end

products excreted by fish (Warren-Hansen, 1982a; 1982b; Solbe, 1982; Porter et al., 1987; Dosdat, 1992a; 1992b; Mommsen & Walsh, 1992; Islam, 2005). The overall mean concentration of ammonium inside the net-cages after 120 minutes of measurement was significantly higher than outside it, suggesting that fish rearing with fish feed input and retarded water flow inside the net-cages may substantially increase the ammonium concentration regardless of whether the fish feed is trash fish or pellet.

During slack water, mean concentrations of ammonium at 120 minutes after feeding were relatively similar among treatments (i.e. trash fish cages, pellet cages, & just outside these cages) suggesting that the water column contained accumulated ammonium trapped within the fish farm and thus its concentration increased. At the same period of study in Jaha, Wong (2002) showed that ammonium concentration were significantly higher within the fish farm (2.88 µmol l^{-1}) compared to outside and away from it (1.44 µmol l^{-1}) and attributed entrapped inorganic nutrient plumes inside fish farms to the close proximity of linearly arranged cage units and adjacent farms which impede tidal flushing and water movement through the cages.

According to McCarthy et al. (1977) ammonium is generally the most biologically available form of inorganic nitrogen for marine phytoplankton and it is vital for the survival of many other aquatic plants. It can be readily taken up by phytoplankton and stimulate their growth (Wu, 1995). Phytoplankton will preferentially use ammonium than other compounds of inorganic nitrogen since it does not need to be reduced (D'Elia & DeBoer, 1978; Valiela, 1995). These further suggest that relatively higher concentration of ammonium inside the net-cages could directly provide nutrient for algal fouling and enhance phytoplankton development which in turn provide food for sessile invertebrates (see also Madin et al., 2009).

The present study shows that nitrate (NO_3-N) was among the important nitrogen

nutrient released from fish rearing activities in floating net-cages. It contributed nearly 33% of the total dissolved inorganic nitrogen nutrient in the fish farm at Jaha. In all tidal phase, leaching rates of nitrate was relatively high from trash fish than in pellet feed particularly during the first 60 minutes after feeding indicating that nitrate was eventually released from trash fish feed similar to that of ammonium. An overall mean concentration of nitrate at 120 minutes after feeding was relatively higher inside net-cages than outside it, suggesting that fish rearing irrespective of the feed type contributes to nitrate enrichment of culture waters.

Nitrate is often considered to be the important nutrient for primary production in the marine environment (e.g. Owens et al., 1989; Fisher et al., 1992). Nitrate containing the lighter isotope is preferentially taken up by phytoplankton particularly during photosynthesis (Holmes et al., 1996). High concentrations of phytoplankton reflect the enrichment of water column with nitrate (Altabet et al., 1995; Ganeshram et al., 1995). In the present study, higher concentration of nitrate along with higher concentration of ammonium could well provide a complete source of nitrogen nutrient directly for algal foulers while enhancing phytoplankton development which in turn serves as food for sessile invertebrates.

In all tidal phases, total concentrations of nitrite (NO_2-N) were relatively minor (i.e. mean concentration $\leq$ 0.50 µmol l^{-1}) or no more than 8% when compared to other inorganic nitrogen nutrient in the fish farm at Jaha. During the slack water, the nitrite concentration increased after feeding (i.e. at 30^{th} minute) especially from trash fish cages suggesting that nitrite leaching occurred during the slow movement of water column. The leaching rate of nitrite during the flood and ebb water was much lower or inconsistent suggesting that water movement and its low stability could reduce its concentration. Nitrite has low stability compared to other dissolved nitrogen nutrients

(e.g. Newton & Mudge, 2005). The overall mean concentration of nitrite after 120 minutes of measurement was much higher inside the net cages given feed than outside it, indicating that fish rearing in floating net-cages also contributes to nitrite enrichment of culture water.

During the flood and slack water, leaching rates of phosphate (PO_4^{-3}) after fish feeding (i.e. at 30^{th} and 60^{th} minute) was much higher in pellet than in trash fish feed suggesting that fine particulates from pellet wastage could release phosphate at faster rates compared to sticky pulp and more variable sizes in trash fish wastage. These suggest that the different sizes and thus surface area of wastage particulates that were released into water column also determine the nutrient leaching rates of pellet (i.e. released fine particulates) and trash fish (i.e. released sticky pulp and variable sizes of particulate) since both type of feeds are of animal sources and thus expected to produce high amounts of phosphorus (see Persson, 1991; De Silva & Anderson, 1995; Sugiura et al., 2000). The results also suggest that pellet feed given to the cultured fishes could stimulate faecal excretion leading to higher concentration of phosphate in the water column. Phosphate has been regarded as the major nutrient associated with caged aquaculture operations which is sourced from faecal production (e.g. Phillips et al., 1985; Molver et al., 1988; Enell, 1987; Folke & Kautsky, 1989; Ackefors & Enell,1990; Hall et al., 1990; Holby & Hall, 1991; Persson, 1992; Hall et al., 1992; Lupatsch & Kissil, 1998).

An overall mean concentration of phosphate after 120 minutes of measurement was relatively similar between pellet and trash fish feed suggesting that both were equally polluting. Their respective concentration was relatively higher than outside the net-cages for all tidal phases indicating that fish rearing in general contributes to phosphate enrichment of culture water. The highest concentrations of phosphate were obtained during the slack water. However, during the flood and ebb water, concentrations of PO^4

inside and outside the net-cages were inconsistent indicating that water flow moved and diluted the leached phosphate in the water column.

High concentration of phosphate in water column of fish culture water is known to contribute to phytoplankton bloom as well as extensive growth of algae and other aquatic plants (e.g. Kato et al., 1985; Phillips et al., 1985; Ambasht & Ambasht, 1992; Beveridge et al., 1994; Cloern, 2001; Islam & Tanaka, 2004). Thus, the relatively higher concentration of phosphate inside the net-cages could encourage extensive growth of algal fouling as well as phytoplankton that will enhance invertebrate foulers as reported in Chapters 3 and 4.

Concentrations of chlorophyll-*a* varied according to the tidal cycle. During the flood and ebb water, total chlorophyll-*a* concentrations were significantly higher inside the net-cages than outside it, however there were insignificant differences during the slack water. This suggests that phytoplankton movement during the flood and ebb flows reduced their concentration outside the net-cages, while generally no or very feeble water movement during the slack water accumulates a higher but similar density of phytoplankton inside and outside the net-cages. Maximum re-suspension of phytoplankton occurred during the maximum flood flow (Bodineau et al., 1998), however rapid flushing will limit phytoplankton accumulation (Cloern, 1996; Eyre, 2000).

Although nutrient is well known to increase the productivity of phytoplankton (e.g. Aure & Stigebrandt, 1990; Wu, 1995; Nixon, 1995; Madariaga, 1995; Downing, 1997; Mallin at al., 1999; Opute, 1999) in the present study, few readings indicate significant correlation between nutrient concentration and chlorophyll-*a* (i.e. inside the net-cages, treatment P or T) suggesting that the leached nutrients during or immediately after feeding, established short, temporary concentration gradients (1 – 2 hours) which are

not likely to cause significant differences in phytoplankton growth. Although, the nutrient gradients are likely reduced due to rapid nutrient absorption by macroalgal biofouling and phytoplankton, growth was not immediately detected. Furthermore, the nutrient gradients are likely disrupted by disparity in water movement inside and outside the cages within the fish farm resulting in "nutrient patches" particularly for NO_3-N and PO_4^{-3} (Wong, 2002). However, the present study did not cater for extensive sampling and hence, correlation between nutrient concentration and phytoplankton production, if any, was not detectable.

Several studies have indicated that nutrient input can be significantly reduced when trash fish feed are replaced by pellet feed because of poor food conversion ratio (FCR) of the trash fish diet (e.g. Ove Arup, 1989; Hansen et al., 1990; Wu et al., 1994; Wu, 1995; Leung, 1996). In the laboratory experiment, Qian et al. (2001) shows the instant release of orthophosphate and ammonia in minced trash fish feed through rapid dissolution of liquid components and feed leftovers regardless of whether or not the feed is consumed by the fish. In the present study, the leaching rate of phosphate was much higher from pellet feed than from trash fish feed, while leaching rate of dissolved nutrient nitrogen from trash fish feed was only slightly higher than in pellet feed. This suggests that the leaching rates of dissolved nutrient in floating net-cages was not only influenced by the type of fish feed input but likely other physical and biological factors such as water movement and the possibility that the leached nutrient is rapidly used up by phytoplankton as well as the biofouling organisms (macroalgae) on cage nettings.

According to Wu (1995) and Doglioli et al. (2004) fish feed wastage however produces mainly organic forms of nutrient, suggesting that fish feed wastage, whether from pellet or trash fish feed, contributes rather to the enrichment of organic particulates than dissolved inorganic compounds inside the net-cages. The present study tends to

support this contention, since such organic particulates serve as food for sessile and especially the mobile organisms (Chapter 3 & 4).

6.4. CONCLUSION

Fish rearing whether with the use of trash fish or pellet feed contribute to the enrichment of NH_3-N, NO_3-N, NO_2-N and PO_4^{-3} in culture water. This enrichment is contributed by fish feed wastage and excretion products of cultured fish. There was however no clear evidence of correlation between nutrient concentration and phytoplankton biomass inside net-cages (T, P) or outside it (O), suggesting that any nutrient gradient established across the cage unit was temporary, due to water movement and perhaps rapid nutrient absorption by phytoplankton and biofouling organisms. The effect of leached nutrient on biofouling development is not clear due to the already higher nutrient concentration inside the farm (compared to outside it). This should be studied by monitoring biofouling on control sites farther away from the farm.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSION

7.1. Biofouling on Floating Fish Cage Nettings in Comparison with Biofouling from Other Substrates

The characteristic of macrofouling assemblages on floating cage aquaculture including their community structure and causing factors of development are thought to be different from that of non-aquaculture because of the difference between netting materials and hard substrates, their floating nature and distance from the seafloor (e.g. Braithwaite & McEvoy, 2005; Cook et al., 2006; Greene & Grizzle, 2007; Madin et al., 2009). Furthermore, the tradition method of macrofouling research to separate the sessile and non-sessile taxa and/or with emphasis on sessile taxa (e.g. Glasby, 2001; Osman & Whitlatch, 2004), also had been suggested to be different for research on cage aquaculture, partly because of the significant roles of non-sessile organisms on fish cage nettings (Braithwaite & McEvoy, 2005; Cook et al., 2006; Greene & Grizzle, 2007; Madin et al., 2009; 2010).

In the present study, there were relatively few species of sessile macrofouling on cage nettings (Chapter 3) when compared to those on hard substrata submerged in subtidal water reported from other studies. For example, from a one-year study of biofouling on an offshore oil platform off Bombay waters, Venugopalan & Wagh (1990) reported more than 100 species of mainly sessile organisms. Qiu et al. (2003) reported at least 32 species of sessile macrofouling on several types of hard substrata such as wood, concrete, steel and tyre after 24 months of submersion in Hong Kong, while Lin & Shao (2002) reported at least 78 species of mainly sessile organisms on several types of steel and concrete submersed for 18 months in Keelung Harbor, Northern Taiwan.

The relatively small number of particularly sessile macrofouling species on cage-nettings is likely due to the pliable and limited attachment surface, as well as shorter immersion time. The fine net comprising filaments of 1.22 mm diameter as used in the present study provided an attachment surface area of 50.26 mm^2 per 12.5 mm net filament length, which is very small and is likely to limit biofouling settlement on it. The encrusting macrofoulers may eventually peel off from the nets due to their increasing weight and water current, thus the long-term effect of immersion time on the 'climax fouling community' may not be apparent on cage nettings. These factors explain why the net macrofouling assemblage was not species-diverse.

According to Venugopalan & Wagh (1990) and Yan et al. (2006), depth is an important source of variation in fouling development. Whomersley & Picken, (2003) indicates that macrofouling diversity on oil platform as well as other solid structure such as mooring will increase with increasing water depth, while Picken (1986) showed a wide bathymetric tolerance of biofouling and the deepest zone was the most diverse in species in an offshore oil platform in excess of 140 m depth. In the present study, the shallow net-cages (1.5 – 2.5 m depth) had a low number of particularly sessile organisms on cage nettings. Cosmopolitan macrofouling organisms such as hydroids, barnacles, bryozoans and mussels which are known to have a wide bathymetric tolerance were represented only by a single species each. Other factors that are likely to contribute to low diversity of sessile macrofouling organisms on cage nettings are the estuarine condition which is subject to salinity fluctuation and the human disturbance due to the removal and cleaning of the fouled net-cages.

Greene & Grizzle, (2007) indicated that colonization and successional patterns of sessile macrofouling assemblages on cage netting material could differ from that of hard substrates. In the present study, cage netting colonization by sessile community began

and became almost entirely dominated by hydroids and bryozoans. On the other hand, barnacles and mussels are often reported to be the earliest dominant colonizers of hard substrata such as rocky shores (Roughgarden et al., 1988), offshore oil platforms (Venugopalan & Wagh, 1990), concrete (Anderson & Underwood, 1994) and steel (Qiu et al., 2003). This suggests that the fine net filaments of cage nettings encourages the settlement and colonization of small encrusting growth forms such as *Plumularia* sp. and *Cryptosula* sp.. Such pioneer species increase the net filament surface area through their growth and colonies, thus providing more space for the attachment and colonization of other macrofoulers.

Cage nettings in aquaculture sites appear to attract a large number of non-sessile species occurring in abundance such as amphipods, isopods, copepods and nematodes. Similar species however occur infrequently in low numbers on hard substrata at non-aquaculture sites (e.g. Abdul Aziz et al., 2001; Lin & Shao, 2002; Qiu et al., 2003; Kashin et al., 2003; Yan et al., 2006). The development (i.e. based on biomass) of non-sessile organisms on cage nettings were significantly ($P < 0.05$) correlated (with a positive correlation) with development of sessile organisms (Chapters 3 & 4). However, these are rarely reported among macrofouling community on hard substrates at non-aquaculture sites (see Lin & Shao, 2002; Qiu et al., 2003; Yan et al., 2006). These further suggest that the enriched food environment of aquaculture site including the microcosm created by the dynamic development of sessile organisms on cage netting is rather important than the type of substrate in attracting a higher population of non-sessile macrofouling in aquaculture site.

The development rates of macrofouling organisms on cage netting in aquaculture site appear to be much higher, i.e. nearly 350 g m^{-2} wk^{-1} inside the net-cages (Chapters 3 & 4) than in non-aquaculture sites. For example, the rate was less than 160 g m^{-2} wk^{-1} as

estimated from the results of Yan et al. (2006) who conducted a study on test panels (300 mm x 200 mm) at varying depths of 3 to 20 m over a 12-month study at a buoy investigation station. This suggests that cage nettings in aquaculture site create an unique man-made environment where a slow water flow regime (i.e. within the fish farm) is surrounded by a fast flow regime (i.e. within the river estuary). The fish cages stop and trap organic particulates in the river system and further provide food to encourage the rapid development of biofouling organisms on them. The cage's wooden platform could provide hard substrate for more stable colonization where a climax community is likely achieved.

7.2. Factors Controlling Biofouling Development in Floating Fish Cage Nettings

Based on the findings of the present study, there are two major factors influencing the development of macrofouling assemblages on aquaculture cage nettings. First, the retarded water flow inside the net-cages and second is the increased availability of organic matter (i.e. feed and feces) and nutrient resulting from fish rearing activities. Sessile and non-sessile macrofouling organisms respond differently to the effects of retarded water flow and enrichment of food and nutrients. It appears that retarded water flow (<10 cm s^{-1}) inside net-cages favors sessile macrofouling which could increase their biomass (g per panel) up to three times higher than in strong water flow (>25 cm s^{-1}) such as outside the net-cages after four weeks of development (see Chapter 4). The retarded water flow along with organic input and higher concentration of nutrients (i.e. NH_3-N; NO_3; NO_2; PO_4) as a result of fish rearing (see Chapter 6) are likely to further enhance macrofouling biomass up to four times higher than in strong water flow i.e. >25 cm s^{-1} outside the net-cages (Chapter 4). Nutrient enrichment could further encourage the growth of fouling algae such as *Polysiphonia* sp., *E. clathrata* and *Lyngbya* sp. as well as phytoplankton blooms which in turn provide food for invertebrates such as *Plumularia* sp., barnacles, anthozoans and mussels (Chapter 3).

The non-sessile organisms are more attracted to the organic input and nutrient enrichment resulting from fish rearing activities, rather than the retarded water flow rate. With organic enrichment, the fouling rate of non-sessile organisms was nearly seven (i.e. 80%) times higher biomass (g per panel) compared to treatments without fish rearing after three weeks of development (see Chapter 4). This suggests that organic enrichment from fish rearing activities could significantly enhance the development rates of non-sessile organisms particularly *Gammaropsis* sp., *Photis* sp. and *Leptognathia* sp.. The type of food essential for these species was however not assessed. They could consume fish feed wastage or rely on the fish feces of cultivated fish. Nonetheless, organic enrichment appears to increase their population significantly on cage nettings. A number of studies have indicated that gammaridean amphipods are sensitive to organic enrichment which reduces species richness but increase their individual abundance (e.g. Tsutsumi, 1987; Rainbow, 1995; Rinderhagen et al., 2000; Pearson & Black, 2001; Mendez, 2002; Bybee & Bailey-Brock, 2003). This is also true in the present study where gammaridean species (i.e. *Gammaropsis* sp. & *Photis* sp.) dominated (Chapter 3).

Outside the net-cages where the flow rate is swifter (>25 cm s^{-1}), the biofouling rate and thus the mean biomass of both sessile and non-sessile organisms was much lower. The fouling assemblages was dominated by *Plumularia* sp. and *Gammaropsis* sp.. The study suggests that strong water flow will reduce the growth rates of bigger forms of sessile macrofouling organisms such as *Polysiphonia* sp., sea anemones *B. amphitrite, E. clathrata* and *X. mangle.* They may experience drag force and shear stress, and reduced retention efficiency of their spores and larvae. Grazing by wild fish and other predators may also affect the development of macrofouling organisms on cage nettings. Several species of wild fish including predators and grazers such as ariid fish and scatophagid fishes are commonly found in a fish farm vicinity of MMFR. This further

illustrate the important effects of disturbance factors such as strong water flow, grazing and food limitation on macrofouling assemblages on cage nettings (see also Connell et al., 1997; Grossman et al., 1998; Witman & Grange, 1998; Madin et al., 2009; 2010).

Other factors such as seasonal fluctuations in estuarine water salinity also influence macrofouling development in aquaculture facilities (Chapter 3 & Chapter 5). A number of studies have indicated that in tropical regions where salinity is greatly influenced by the monsoonal rain, lower salinity can have detrimental effects on marine organisms (e.g. Ansari et al., 1984; Reddy & Hariharan, 1985; Kondalarao & Murty, 1988; Ansari & Parulekar, 1993). Thus, the low abundance of macrofouling during the wet season is features of stressed species at lower salinity which may adapt to live but with reduced growth rates or enter a dormancy state (Chapter 3). On the other hand, low salinity may result in the propagation of 'wet season' dominant species that could tolerate a wider range of salinity fluctuations.

The seasonal variation in biofouling population is also thought to be the result of a combination of factors including salinity and other abiotic and biotic factors that affect macrofoulers directly or indirectly (Chapter 5). This suggests that the seasonal availability of natural food such as phytoplankton and nutrients, and larvae, spores and juveniles of macrofouling organisms also contribute to the variation of their populations on cage nettings during the dry and wet seasons (see also Underwood & Anderson, 1994; Madin et al., 2009).

7.3. Role of Sessile Versus Non-sessile Biofouling Organisms

Development by non-sessile organisms on cage nettings appears to be related to the development of sessile organisms themselves. Indeed, the succession of species of amphipods on the cage nettings which was in tandem with the sessile assemblage has been demonstrated (Chapter 3). The relatively higher population of non-sessile

organisms found inside the net-cages, e.g. amphipods and tanaids, as compared to outside the net-cages, may be their attraction to the microcosm created by the sessile assemblage that provides food, habitat space, and shelter (Madin et al., 2009). For example, the majority of common seaweeds are important food source for gammarid amphipods which often select their habitat on/near seaweeds; amphipod reproduction and biomass are often correlated to the abundance of seaweeds (Lewis & Stoner, 1983; Lewis, 1984; Costa & Costa, 1999; Cruz-Riveira & Hay, 2000a, b; Duffy, 1990; Pavia et al., 1999; Nelson, 1980; Stoner, 1980; Klumpp & Kwak, 2005; Detwiler et al., 2002). Hence, algal fouling on cage nettings such as by *Polysiphonia* sp., and *Enteromorpha clathrata* is likely to provide food for amphipods.

Among the role of non-sessile organisms (such as amphipods) inside the fish net-cages is their reworking of organic waste and enhancing the release of nutrients for sessile macrofouling organisms such as macroalgae. Many small epifauna including amphipods are ecologically important in facilitating nutrient cycling (Harrison 1977; Bartodzieg, 1992; Myers, 1997). Furthermore, the constructed silty tubes of amphipods facilitate the attachment and growth of sessile macrofoulers such as *Polysiphonia* sp., *Enteromorpha clathrata* and *Lyngbya* sp. as well as strengthening the byssus attachment of *Xenostrobus mangle.* Hence, mobile organisms on cage nettings should be considered as an important component of macrofouling because of their significant activity and biomass on cage nettings (Madin et al., 2009; 2010).

7.4. Biofouling Prevention and Control in Floating Net-Cages

One of the reasons for conducting this study is to generate information leading to the prevention and control of biofouling on cage nettings. The suggested methods and consideration to control or at least minimize biofouling impact on cage netting of aquaculture in a tropical estuary are discussed below.

7.4.1. Siting, Design and Arrangement of Cage Units

From the finding of the present study, it is recommended that feasibility study on the local area hydrodynamics should be conducted at the designated aquaculture site. It would be advantages for the fish farming area to have better water flow that could help reduce macrofouling rates as well as the dispersion of fish farm debris. If the flow rate inside the net-cage could be kept above 25 cm s^{-1}, biofouling would be greatly reduced (Chapter 4). The flow rate before impacting the nets varied from 30 cm s^{-1} – 48 cm s^{-1} based on a progressive net impedance of 20 – 75 – 90% as the water flows through three serial clean net-cages (based on Figure 4.1). Hence, the incoming flow rate should be at least above 50 cm s^{-1} to ensure a flow rate of >25 cm s^{-1} through three adjacent clean nets (Madin et al., 2010).

To further improve flow-through, the design of the floating fish farm and thus the arrangement of each cage units should be studied particularly its interaction with tidal hydrodynamics. For example, the existing distance of less than 0.5 m along both sides of linearly-linked net-cage array should be increased to improve water movement throughout the farm. Net-cages should not be serially arranged in tidal estuaries where the dominant flow is bi-directional. Instead, the net-cages should be so arranged to increase the surface area of contact with the incoming current (Figure 7.1). Since fingerlings are enclosed by small mesh size (1.6 cm) net-cages and therefore subjected to higher biofouling rates, these cages are preferably located around the farm's periphery so as to receive maximum flow rates during flood and ebb water. Net-cages stocked with matured fish have greater mesh size and therefore lower biofouling rates; they should preferably be positioned within the area of slower water movement in the farm.

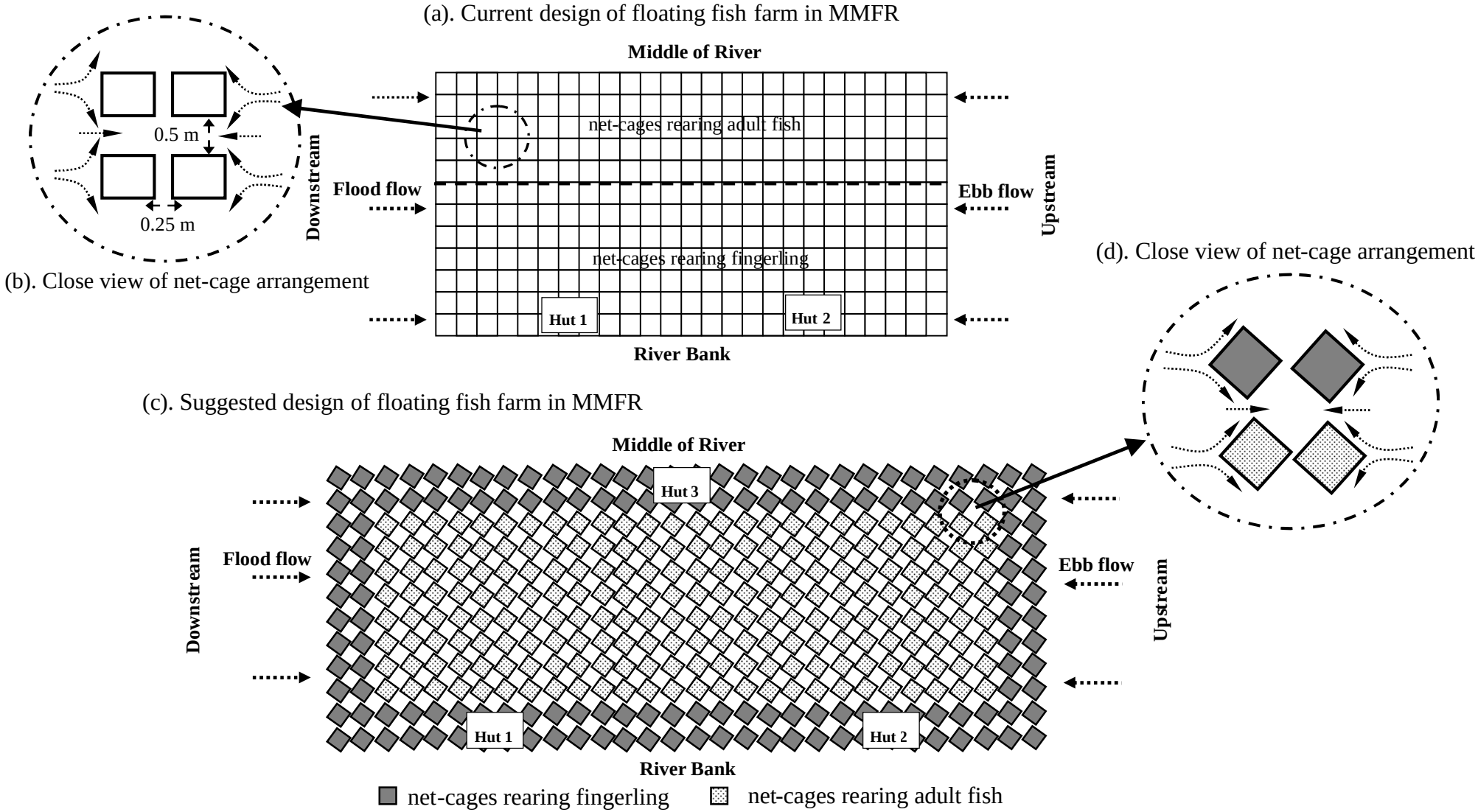


Figure 7.1. Sketch diagrams of (a) current design of floating fish farm where net-cage units arranged in serial position with each other resulted in the reduced water flow throughout them (as indicated by dash arrow), see close view (b). Suggested design of floating fish farm base on current findings (c). Each cage units arranged with empty space between each other thus allow more water flow (as indicated by dash arrow) through them, see close view (d).

7.4.2. Rearing Season

Based on the findings of the present study, development rates of macrofouling are significantly higher during the dry season than in the wet season (see Chapter 3). Thus, it is recommended that the rearing of young fish in fine-meshed cage-nets be carried out during the wetter months in order to reduce the frequency of net cleaning for five weeks (See section 3.3.2.). Thus, the ideal period of rearing young fish is during the heavier rainfall periods which normally occurs during March and April, and during November and December (see Figure 3.1); these periods are coincident with lower salinity (i.e. < 20 ppt) shown to reduce the development rates of biofouling (Madin et al., 2009).

7.4.3. Biological Control on Biofouling Organisms

Many fish and invertebrates are known to feed on and remove other organisms such as sessile benthos naturally from hard substrata (e.g. Hawkins & Hartnoll, 1983; Van der Veer et al., 1998). 'Biological control' aims to use or exploit these natural grazers or predators to control or at least to minimize the impact of biofouling in aquaculture (e.g. Hidu et al., 1981; Van der Veer et al., 1998; Enright et al., 1983; Cigarria et al., 1998; Ross et al., 2004). This method is thought to be an environmentally sustainable way to control biofouling population that avoids the use of chemicals and relatively involves a minimal cost (e.g. Ross et al., 2004). Furthermore, the biological control organism that is exploited can be used as a form of polyculture (e.g., Littlewood, 1990; Ahlgren, 1998; Ross et al., 2004).

Based on findings of the present study, biological control on macrofoulers should be emphasized on sessile organisms that contribute almost 90% of biofouling biomass on cage nettings (Chapter 3). *Plumularia* sp. which is important as the earliest and dominant colonizer throughout the fish culture period, along with vigorously growing algal species (i.e. *Polysiphonia* sp. and *Enteromorpha clathrata*) at the surface should

be the main concern when applying biological control methods in floating net-cages. Thus, the biological control should be both efficient in controlling invertebrate and algal fouling. Among the biological control methods that have been proven to reduce hydroids and algal fouling as well as bryozoans in aquaculture are the use of sea urchins such as *Echinus esculentus* and *Psammechinus miliaris* (Ross et al., 2004; Lodeiros & Garcia, 2004). Other species that remove hydroids are hermit crabs (*Pagurus* spp), top shell crab (*Calliostoma zizyphinum*) and fish (*Fundulus heteroclitus*) (Enright et al., 1993; Flimlin & Mathis, 1993; Skjaeggestad, 1997; Ross et al., 2004), while grazing gastropods such as periwinkles (*Littorina littorea*) remove algal fouling (Enright et al., 1983; Skjaeggestad, 1997; Cigarria et al., 1998; Lodeiros & Garcia, 2004).

The seasonally abundant macrofoulers such as barnacles, mussels and anemones can be controlled by a combination of sea urchins, dog whelks (*Nucella lapillus*), and predatory fish such as cunner and wrasses that could remove both hard and soft fouling on nets (e.g. Hidu et al., 1981; Minchin & Duggan, 1989; Kvenseth, 1996). Currently there is no suggested method to reduce mobile macrofoulers such as amphipods in aquaculture, however predatory fish that feed on these organisms such as cunner and wrasses might be useful to reduce their population on cage nettings (e.g. Harris, 1986).

7.4.4. Control of Biofouling Enhancer

Other possible solutions to minimize biofouling rates on cage netting are to introduce effective biological control organisms that could remove or reduce biofouling enhancers such as organic matter including uneaten fish feed particulates, fish faeces, metabolite waste as well as natural food such as phytoplankton inside the net-cages. The accumulated waste materials that settle on net filaments provide increased attachment surfaces for sessile macrofoulers and important substrate building material for the construction of silty or living tubes by mobile macrofoulers such as tubicolous

amphipods. Furthermore, the waste materials which mainly accumulated at the cage bottom also contributes significantly to the heavier weight of fish cages.

Suggested methods to reduce biofouling enhancers in aquaculture, include the use of filter-feeding macrofauna such as scallops and clams which are known to remove organic particulates including phytoplankton, organic and inorganic particles in aquaculture farm (Littlewood, 1990; Shpigel et al., 1993; Ahlgren, 1998; Mazolla & Sara, 2001; Ross et al., 2002; 2004). Furthermore, a number of studies on the gut content of wild fish congregating around salmon cages in European waters show that they consume the uneaten food pellets, faecal and other waste material derived from culturing activities (Carss, 1990; Papoutsoglou et al., 1996; Black, 1998; Johansson et al., 1998; Pearson & Black, 2001; Felsing et al., 2005). These studies suggest that filter-feeding macrofauna and wild fish can be used and exploited as biological tools to reduce waste material and other natural food available for macrofoulers inside the fish cage.

7.4.5. Biofouling Control with Nontoxic Material

The use of most commercially available, antifouling chemicals or coatings on cage nettings is largely restricted due to concern of environmental toxicity as well as consumer preference that may jeopardize the market image of cultured fish (Wu, 1995; Hodson et al., 1997; Champ, 2000; 2001; 2003; Braithwaite et al., 2007). Therefore, less environmentally harmful material to control biofouling is now being explored around the world (e.g. Holmstrom & Kjelleberg, 1994; Armstrong et al., 1999; Harder & Pei-Yuan, 2000; Kjelleberg & Steinberg, 2001; De Nys & Steinberg, 2002; Bhadury & Wright, 2004). This includes the manipulation of antimicrobial compounds on the larvae or spore of some crustaceans and seaweed to prevent the settlement of macrofouling organisms (Gil-Turness et al., 1992; Holmstrom et al., 1992; Tatewaki et

al., 1983; Clare, 1996). However, the natural anti-foulants are still the expensive products and comprehensive solution has yet to be achieved. Thus, there remains considerable impetus for the development of more environmentally sustainable methods such as the use of biological control (e.g. Ross et al., 2004)

7.5. Future Research on Biofouling of Cage Nettings to Improve Water Quality

❖ As pointed out by Ross et al. (2004), despite promising results, biological control is underdeveloped and there is a need for more research. Although the use of grazers such as scatophagid fish to reduce algal fouling is commonly practiced by fish farmers in MMFR, extent of its effectiveness need to be explored. Thus, future research should be conducted on the use of suggested biological controls organisms such as sea urchins, hermit crabs and other grazers.

❖ Since macrofouling can also be associated with high concentration of plankton and organic detritus (e.g. Ross et al., 2002), further study on the potential use of filter feeding macrofauna such as mussels and oysters in tropical net-cages so as to minimize macrofouling enhancer should be carried out. In such instances, the biological control organisms such as mussels might reduce macrofouling rates from outside the net-cages by competing for their suspended food while increasing mussel growth. Polyculture would further increase the profitability of fish farm.

❖ Future study on the general design of floating fish farm and its interaction with flow hydrodynamics in the estuary to maximize water flow rates throughout the farms area should be carried out. The net-cage arrangement in the farm, design of net-cage units (e.g. shape) net colour, mesh size and material also require further studies to reduce macrofouling and improve water quality.

❖Clogging rates of net cage opening and how they affect water flow rates, oxygen supply and waste material dispersion within the fish farm is another important research

area. Quantitative data on the clogging rate is likely more useful for fish farmers than the use of total wet biomass data when conducting a net cage change and cleaning. Furthermore, this type of study is probably useful when applying antifouling coatings so as to estimate their durability upon use. This study can be conducted by calculating the net aperture occlusion with the aid of an image capture-and-analysis system (Braithwaite et al., 2004).

❖ Research on the use of natural antifouling compounds as an alternative to toxic antifouling compound has been ongoing but promise little in term of their commercial application. This includes the development of antifouling coatings using biogenic compounds or secondary metabolites that are present in various marine organisms and function as natural anti-settlement agents.

❖ More studies on the role of sessile and non-sessile macrofouling organisms particularly their feeding behaviour inside the net-cages are required so as to determine their role in the utilization of feed and waste material derived from aquaculture activities. This study also has great significance in academic terms for elucidating the interaction processes of macrofouling community development and organization in a specific aquaculture ecosystem.

7.6. Summary

This study has elucidated the community structure and short-term colonization dynamics of macrofouling assemblages on fish cage nettings and the factors that influence their development (Figure 7.2). There were 35 species of macrofoulers found on the cage nettings in the tropical estuary. Macrofouling of cage nets is year-round and consistent, but the dry season (higher salinity) favors higher biofouling rates with increased species-specific abundance. Sessile macrofouling on cage nettings is generally not depth-dependent but intense competition among organisms causes species to be

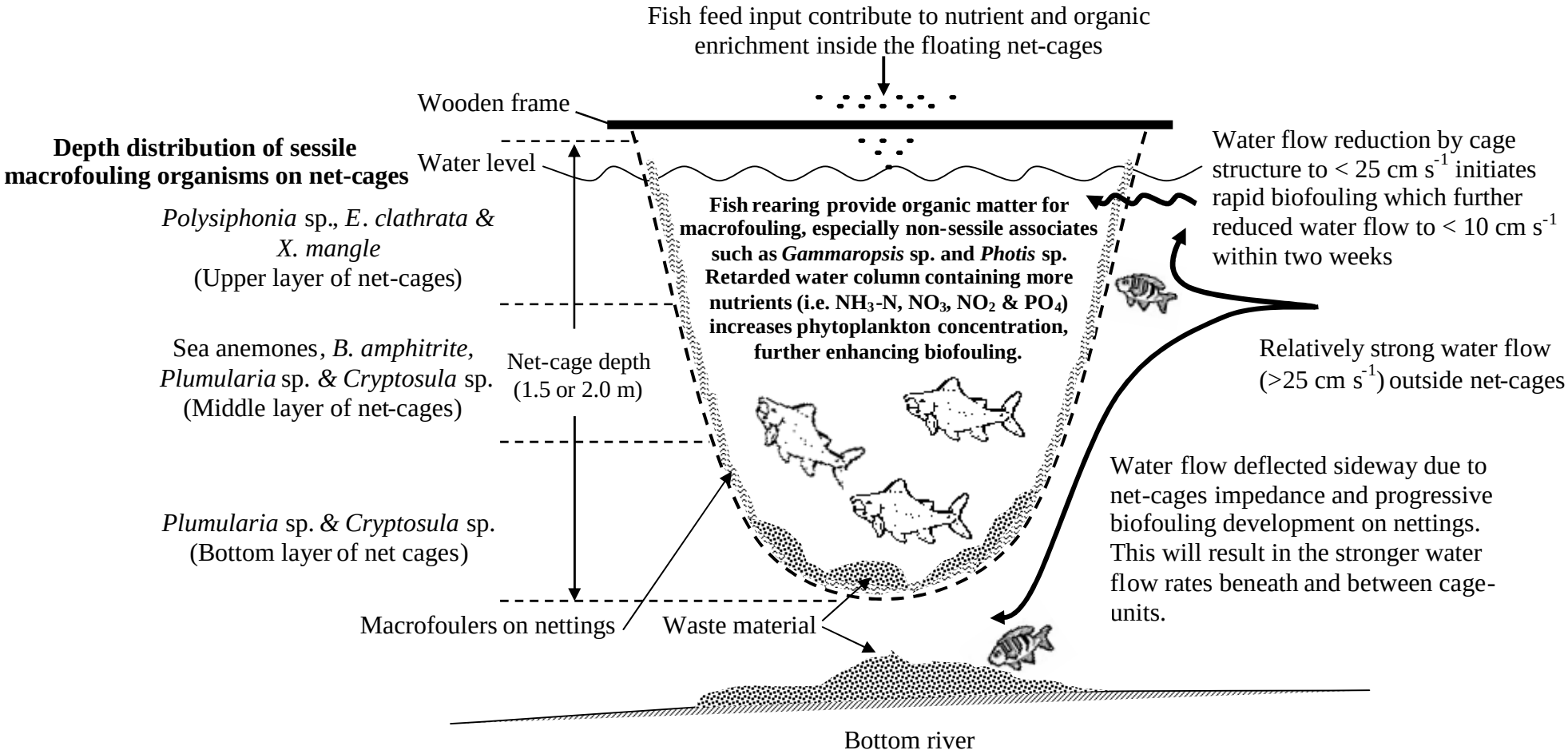


Figure 7.2. Schematic diagram showing the main findings of study on how fish rearing, feed input and water flow attenuation contribute to the macrofouling of floating net-cages.

concentrated at particular depths. Species depth distribution appears to be seasonally different due to their different responses to the seasonal salinity variation. The sessile macrofouling community generally begins with the encrusting *Plumularia* sp. (a cnidarian), while the non-sessile macrofouling community begins with *Gammaropsis* sp. (an amphipod). The colonization rates of macrofouling organisms are generally influenced by site, season, fish rearing activity and water flow rates. Decreased water flow (< 25 cm s^{-1}) through clean net-cages will initiated rapid sessile macrofouling organisms whether with or without organic enrichment from fish rearing. Biofouling itself further reduced water flow to below <10 cm s^{-1} within two weeks. On the other hand, non-sessile organisms appear to be attracted to cage nets due to organic enrichment from fish rearing (Madin et al., 2009; 2010). Overall, this study concludes that water flow attenuation in floating net-cages is the most important factor contributing to the abundance of sessile organisms which make up almost 90% of the total fouling wet by weight. To reduce the biofouling problem, it is recommended that a sufficiently strong (> 50 cm s^{-1}) tidal flow be present before siting a floating net-cage farm in an estuary dominated by bi-directional tidal flow. The rearing of young fingerlings which requires fine-mesh net-cage should preferably begin during the wetter months and their cages should be located around the farm's periphery so as to receive maximum flow through. These steps should reduce macrofouling rates and the frequency of net cleaning. Future studies on the feasibility of other methods to reduce biofouling and improving water quality in cage culture are also recommended.

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APPENDIX 1

Effects of Fish Feed on Biofouling Development in Floating Fish Cages

Madin, J. and Chong, V.C. (2004). Effect of fish feed on biofouling development in floating fish cages In: Marine Science Into the New Millennium; New Perspectives and Challenges, Proceedings of the Asia-Pacific Conference on Marine Sciences & Technology, 12–16 May 2002, Kuala Lumpur, Malaysia (ed. S.M. Phang, V.C. Chong, S.C. Ho, N. Mokhtar & L.S. Jillian Ooi), University of Malaya, Kuala Lumpur. pp. 307–324.

Effects of fish feed on biofouling development in floating fish cages

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Abstract

The effects of feeding fish with formulated pellets and trash fish on biofouling development in floating cage nets were studied in the Jaha and Sangga Besar estuaries located within the Matang Mangrove Forest Reserve, Malaysia. The former estuary contained fish cages of low density (300 cages), while the former contained cages of high density (> 6,000 cages). The two term studies were conducted during the dry season between August and September 2000 and wet season between November and February 2001. The experimental design comprised three treatments: pellet feed, trash fish feed and control (without any type of feed, i.e. outside fish cage), with 3 replicates each (different cages). Eight (dry season) or 12 (wet season) nylon net panels each of 0.2 m × 2 m dimension and of 1"-mesh size were placed inside the experimental fish cages. Biofouling rates on the net panels were then followed every week by removing one panel per week from the experimental cages. Two groups of biofouling organisms, attached and free-swimming forms, were studied. Results indicated that during the dry season, biofouling increased rapidly during the first four weeks, reaching near maximum (wet) biomass of 456.9 g and 43.8 g per panel at the fourth week for attached and free-swimming forms, respectively. While the mean biomass of attached biofoulers were significantly higher ($p < 0.01$) in Sangga Besar (351.5) than in Jaha (307.9 g), there was significant difference ($p > 0.05$) for free-swimming biofoulers (29.4 g and 28.7 g, respectively). However, for both estuaries, the mean biomass of attached and free-swimming biofoulers for the three treatments were significantly higher ($p < 0.001$) in the following order: Pellet > Trash feed > Control. During the wet season, net biofouling in Jaha reached a maximum biomass of 512.9 g after 11 weeks for attached forms and 32.5 g for free-swimming forms at the twelveth week. However, during the dry season biofouling by both attached and free-swimming forms were significantly ($p < 0.001$) higher (1.6 and 1.9 times higher, respectively) than during the wet season. The effects of feed treatments on biofouling were as follows: Pellet = Trash fish > Control ($p < 0.01$), for both attached and free-swimming forms. These experimental results suggested that the physico-chemical parameters such as salinity, dissolved oxygen, water current speed and turbidity could affect biofouling development. Use of quality feed of higher water stability with better binding agent (less powder) could reduce the rapid growth of suspension or filter feeders such as barnacles and bryozoans. Net biofouling management should include use of appropriate net thread material and surface, net mesh size, location and season.

Introduction

The aquaculture industry is fast becoming an important sector meeting the increasing demand for fish. Its rapid growth in the Asia Pacific region has led to concern of its negative impacts on the environment (Gowen and Bradbury, 1987; Frid and Merar, 1989; Aure and Stigebrandt, 1990). In Malaysia, in view of the stagnating capture fishery, aquaculture is expected to become more important as a major source of readily available seafood and protein. The development of

mariculture using floating net cages, rafts, fish pens and net enclosures encounters a similar major problem of biofouling as faced by the shipping industry. Biofouling incurs yearly losses of over US$6.5 billion to the global shipping industry mainly from higher fuel consumption and regular maintenance involving cleaning and painting of ship hulls (Blanke and Yang, 2001; Callow and Callow, 2002).

Biofouling organisms can be grouped under two main categories, microfoulers and macrofoulers. The microfoulers such as algal spores, diatoms, marine bacteria, etc. form the primary organic film, while the macrofoulers are encrustations such as algae, cnidarians, barnacles, bryozoans and mussels (Ferguson Wood, 1950). All fouling even in natural seawater begin with spontaneous deposition of primer coat of natural, high polymer film. The film component are most often derived from the trace background amounts of decaying animal and vegetable matter - in essence, the "humic acid of the sea" (Baier, 1999). Bacteria colonize within hours, as may unicellular algae and cynobacteria (blue-green algae). These early small colonizers form the biofilm, an assemblage of attached cells sometimes referred to as 'microfouling' or 'slime'. Macrofouling community (consisting of either soft or hard fouling) then develops and overgrows the microfouling community.

The most widely recognised impact of fish cage aquaculture is organic and inorganic enrichment, since a large portion of the fish food is uneaten, and becomes deposited on the seabed or dispersed in the water column as dissolved nutrients or particulates (Qian *et al.*, 2001). Many fish farms are heavily fouled with microfoulers and macrofoulers that consume dissolved nutrients (e.g. algae), filter feed (e.g. barnacles) or degrade organic particulates (e.g. bacteria). One method suggested to reduce fouling rates in fish cages and nets is in fact to introduce competing filter-feeding macrofauna, such as scallops and mussels, to remove organic particulates, including phytoplankton, and thus removing both organic and inorganic pollutants (Shpigel *et al.*, 1993). The positive impact of biofouling in terms of removing farm organics and pollution is unknown. However, biofouling is a bane to cage farmers because its management, including cleaning and net damage, is added production costs. Moreover, biofouling causes significant reduction of water flow through net cages, often resulting in serious aeration and water quality problems.

The fish cage culture industry in Malaysia is a relatively recent development with large scale farming in marine waters taking off only in the 1980s (Shariff and Gopinath, 1999). In 1995, fish cages constituted only 75 ha as compared to approximately 2,620 ha of shrimp ponds; however, the rate of increase in fish cage farming, which is more productive than pond culture, is nearly six times faster than the growth of pond shrimp cultivation (Chong, 1998). In 1997, there were 58,500 cages in the country with total area of 680,893 m^2 and total production from cage farms amounted to 7,314 tonnes or 8% of the total aquaculture production. Production was oriented largely towards production of high valued finfish for the trade. The main finfish reared is the sea bass (*Lates calcarifer*), groupers (*Ephinephelus* spp.) and mangrove jacks (*Lutjanus* spp.) (Shariff and Gopinath, 1999).

In order to develop an efficient and cost-effective method to prevent or control biofouling, more studies on the biology and ecology of biofouling organisms are necessary. This paper presents the preliminary results of a study carried out to investigate the development of biofouling community on floating fish cages as affected by the type of fish feed given to the cultured fish.

Material and Methods

Study site

The study was carried out at two cage culture farms located in the estuaries of the Jaha and Sangga Besar rivers which are located within the Matang Mangrove Forest Reserve, Malaysia. Low density fish cages (ca. 300 cages) are located in the Jaha while high density cages in total 6,564 cages covered 5.3 ha of the estuary of Sangga Besar and its tributary with the Selinsing River (**Fig. 1**).

Experimental design

Two studies were conducted during the dry season between August and September 2000 and another during the wet season between November 2000 and February 2001. The experiment design comprised of using a total of six cages given three treatments: pellet feed, trash fish feed and control (without any type of food, i.e. outside fish cages), with three replicates each (different cages). Each cage was stocked with 200 pieces of seabass (*Lates calcarifer*) which were fed daily at the rate of 0.60 - 0.75 kg of dry pelleted feed or 1 - 2 kg of trash fish. Surrounding (non-experimental) cages of between 100 - 150 cages in the farm were fed a total of between 300 - 800 kg of trash fish daily by the farmer, depending on the tide.

Eight (dry season) or 12 (wet season) nylon net panels each of 0.2 m × 2.0 m dimension and of 1"-mesh size (**Fig. 2**) were placed inside the experimental fish cages. Biofouling rates on the pre-weighed net panels were then followed every week by removing one panel each week from the experimental cages, until completion of the experiment. Net panels were immediately placed in 3-l bottles with buffered 10% formalin as preservative. In the laboratory, each net panel was gently agitated, removed from its bottle and weighed. The difference in weight before and after the experiment represents the weight of the attached macrofoulers. The net panel was then returned to its bottle with fresh 5% formalin for further analysis. Free-swimming microbiofoulers which had dropped to the bottle bottom was collected by sieving the entire fluid through a 125 μm-mesh Endecott sieve and washing with running tap water to remove sediments. The microfoulers were washed onto a preweighed wire gauze of the same mesh size, before their combined wet weight was determined. Samples were then resuspended in 70% alcohol solution for further analysis (results not reported here).

Physico-chemical parameters

Temperature (°C) dissolved oxygen (mg l^{-1}), turbidity (NTU), salinity (ppt) and pH were determined at the surface and bottom by using an YSI 3800 multiparameter sonde and meter. Water current (ms^{-1}) and direction (bearing) were measured by an electric water current meter (Toho Dentan model CM-2).

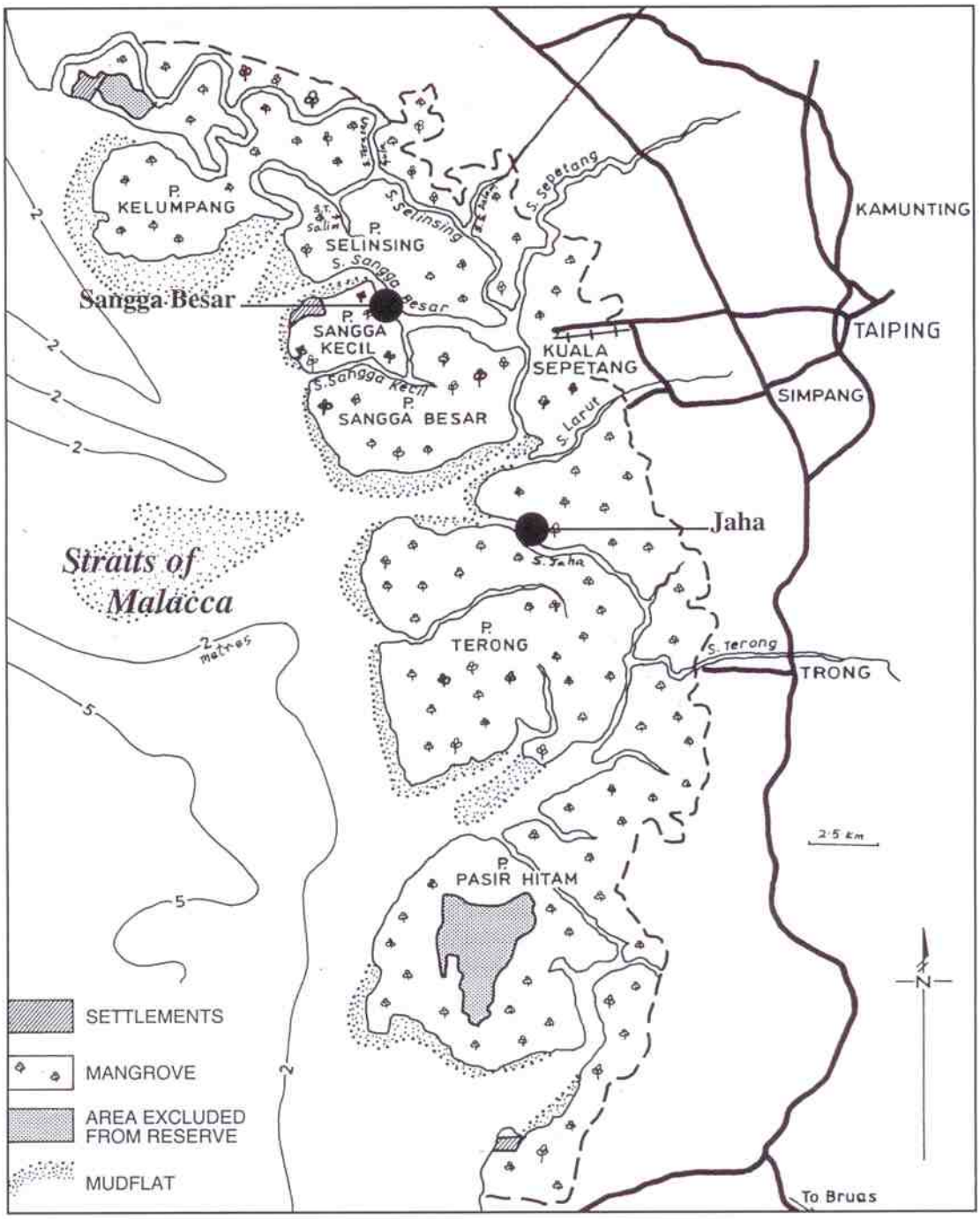
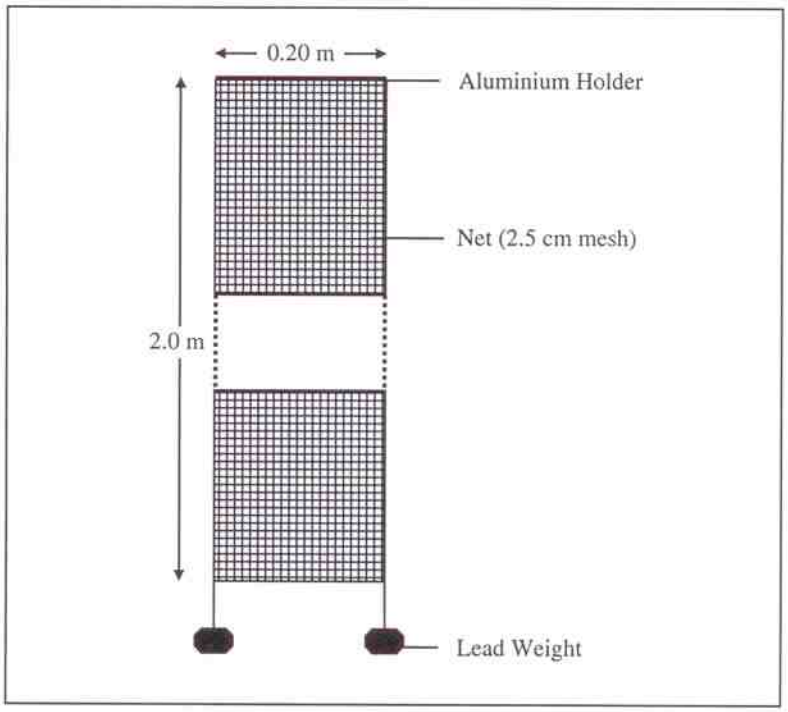
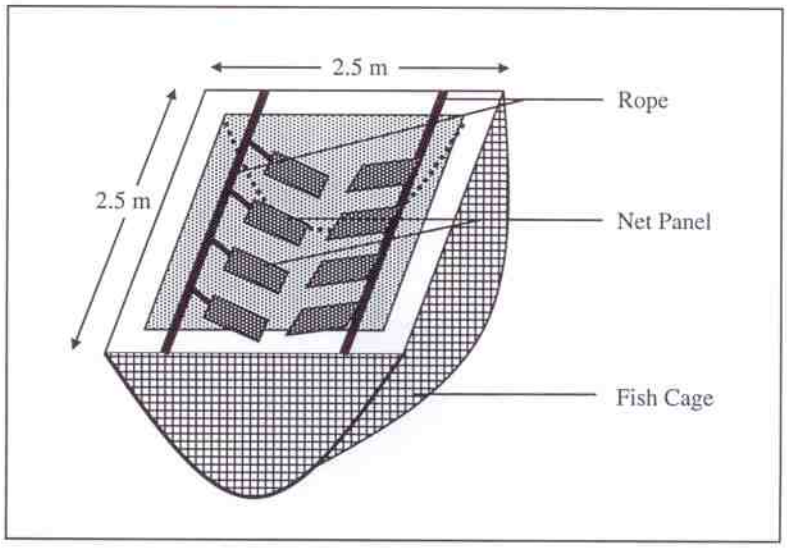


Fig. 1. Location of study site (Jaha and Sangga Besar) in the Matang Mangrove Forest Reserve, Perak, Peninsular Malaysia. Adapted from Sasekumar *et al.* (1994).



(a) Single net panel.



(b) Several panels positioned inside an experimental fish cage.

Fig. 2. (a) Sketch diagram of experimental net panel, and (b) how they were positioned inside the fish cages.

Statistical analysis

ANOVA (3-way) was carried out to investigate the significant effects of feeds (pellet*trash fish*control), week (1, 2, 3...8) and estuary (SJ*SSB) on biofouling (attached and free-swimming) biomass during the dry season. Another (3-way) ANOVA was carried out to investigate effects of season (wet*dry), week (1,2,3... 12) and feed on biofouling biomass in SJ. The Student Newman-Keuls test was used for multiple comparisons of the means.

Results and Discussion

Two groups of biofouling organisms, attached and free-swimming forms, were studied. The attached forms include Hydroids, Gastropoda, Bivalvae, Gracilaria, Barnacles and Byozoans. The organic or mineral matter was found to be associated with the, attached biological fouling organisms. The mobile biofoulers on net panels mostly belonged to phyla Arthropoda, Annelida and Nemertina. During the dry season, the mean biomass of attached biofoulers were significantly higher (main effect, $p = 0.0013$) in Sangga Besar (351.5 g) than in Jaha (307.9 g). For both rivers, the biofouling biomass were significantly different among weeks, increasing with time ($p < 0.0001$). For both rivers, the mean biomass of attached biofoulers for the three treatments were significantly ($p < 0.0001$) different in the following order: Pellet > Trash feed ($p < 0.05$) > Control (for all comparisons $p < 0.05$). There were however significant interaction effects between feed and estuary ($p < 0.001$) (**Fig. 3**).

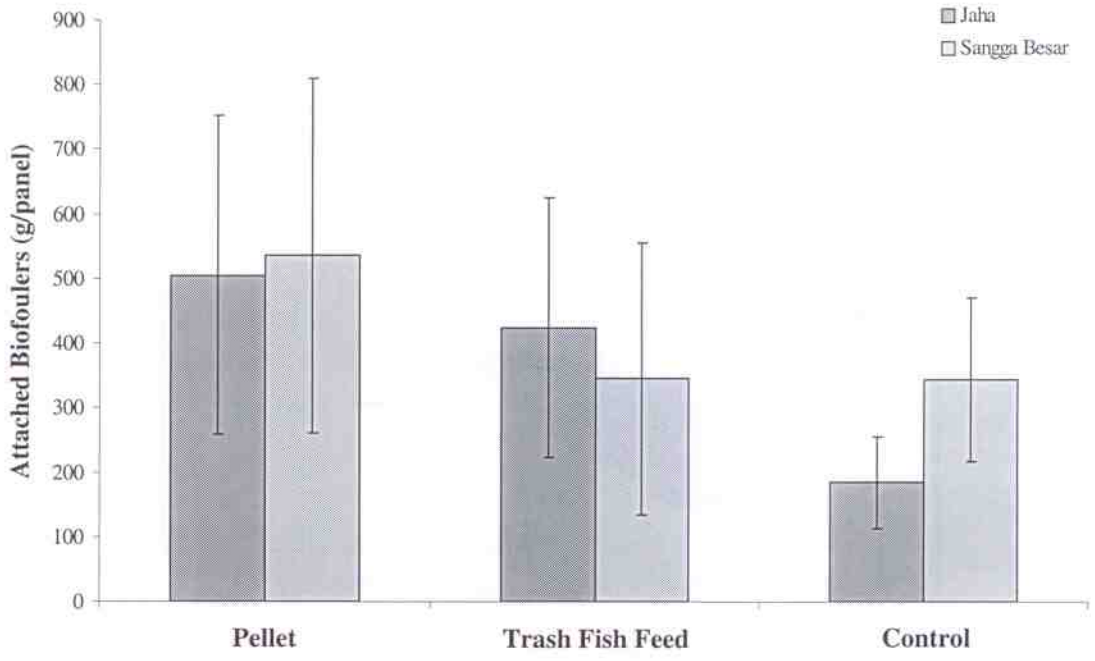


Fig. 3. Effects of fish feed and estuary (Jaha and Sangga Besar) on the mean biomass of attached biofoulers on experimental net panels. Vertical line on bar indicates standard deviation.

The mean biomass of free swimming biofoulers were however not significantly different ($p = 0.58$) between Jaha and Sangga Besar with values of 29.4 g and 28.7 g, respectively (**Fig. 4**). Biofouling biomass increased with time ($p < 0.0001$). As in the attached type of biofouling, the effects of feed treatments on the biomass of free swimming biofoulers in both rivers were significantly different ($p < 0.001$) in the following order: Pellet > Trash feed > Control (for all comparisons $p < 0.001$). Except between week and river, all interaction effects among factors were significant (see **Fig. 3** for interaction effect between feed and estuary).

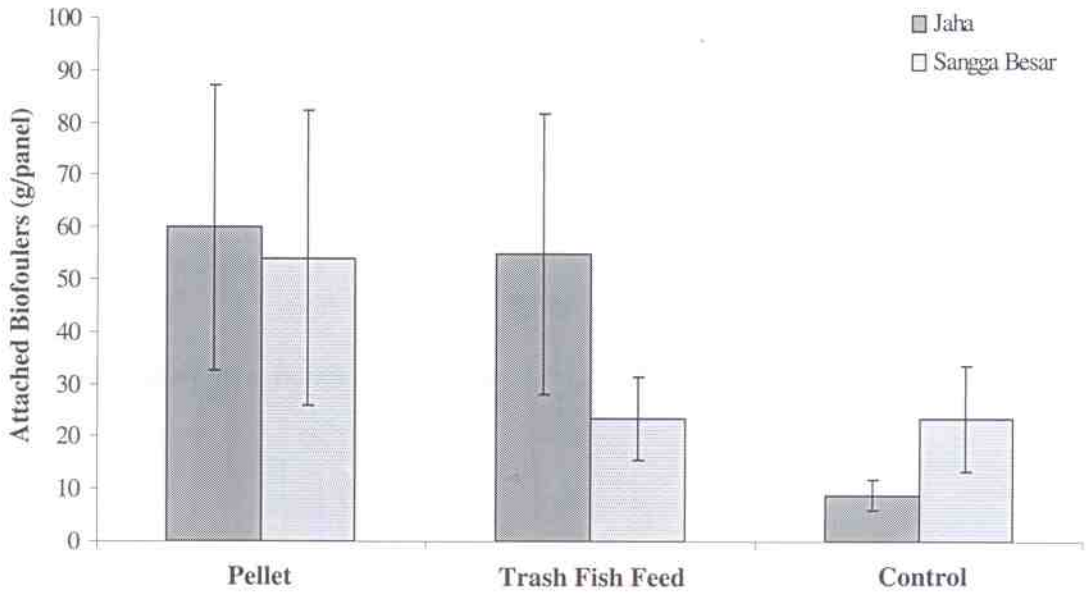


Fig 4. Effects of fish feed and estuary (Jaha and Sangga Besar) on the mean biomass of free-swimming biofoulers on experimental net panels. Vertical line on bar indicates standard deviation.

The results indicated that during the dry season, biofouling in both rivers increased rapidly during the first four weeks, reaching near maximum (wet) biomass of 456.9 g and 43.8 g per panel for attached and free-swimming forms, respectively. During the wet season, net biofouling rates (which were monitored in Jaha only) were slower, reaching a maximum biomass of 512.9 g per panel after 11 weeks for attached forms and 32.5 g per panel for free-swimming forms at the 12th week (**Fig. 5**). Both types of biofoulers were significantly ($p < 0.001$) higher in biomass during the dry than wet season. In Jaha, the biomass of attached biofoulers during the dry and wet seasons was 307.9 g and 188.9 g per panel, respectively, while free-swimming biofoulers were 29.4 g and 15.6 g per panel, respectively. The effects of feed treatments on attached biofoulers were as follows: Pellet > Trash fish > Control (all $p < 0.05$), and on free swimming biofoulers: Pellet = Trash fish > Control (at $\alpha = 0.05$), for both dry and wet seasons (**Fig. 6 and Fig. 7**).

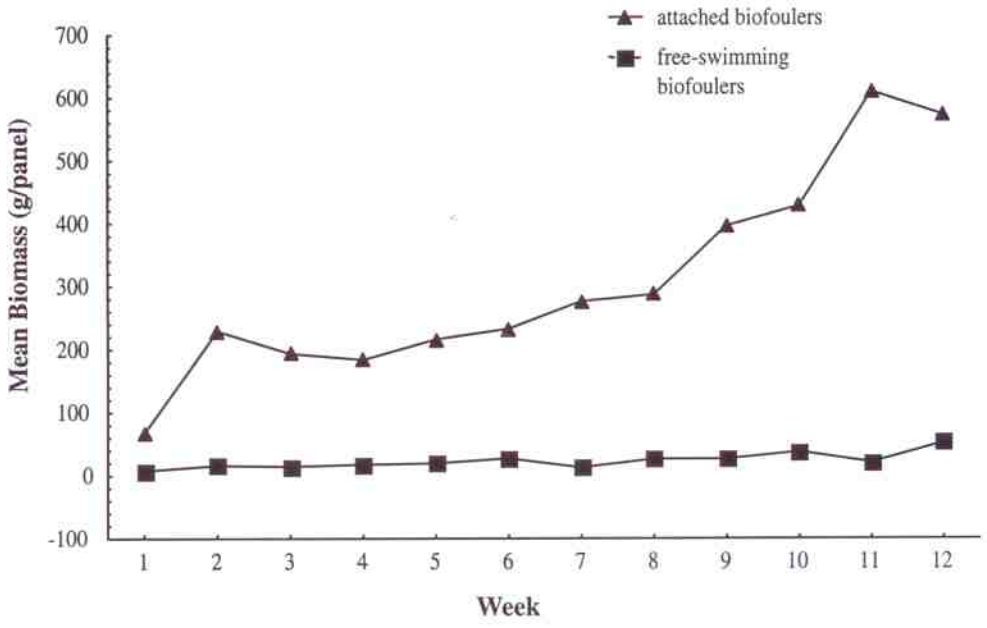


Fig 5. Mean biofouling weight (g) of attached and free-swimming biofoulers in Jaha estuary during the wet season.

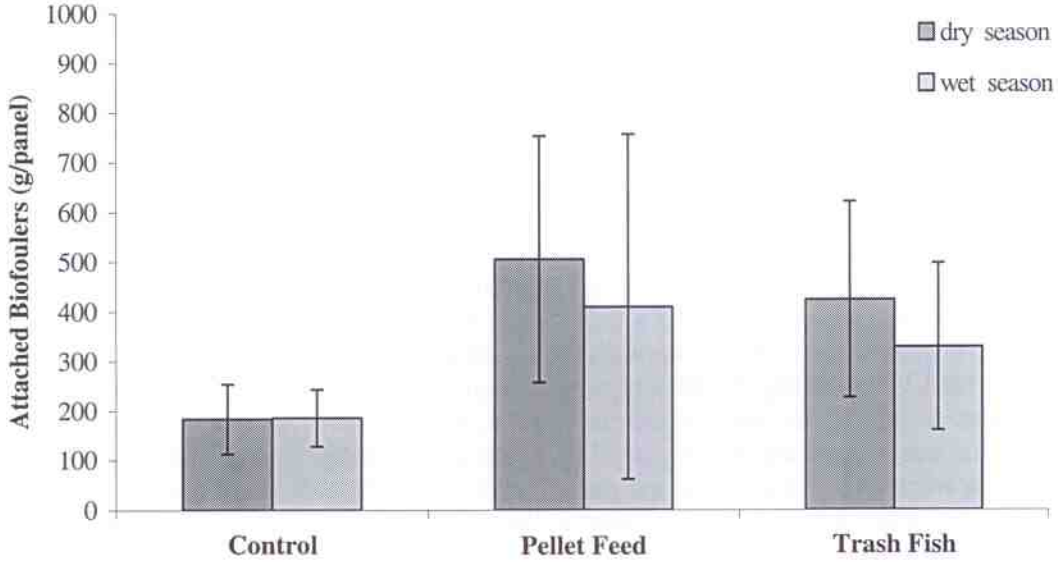


Fig. 6. Effects of fish feed and season on the mean biomass of attached biofoulers on experimental net panels at Jaha estuary. Vertical line on bar indicates standard deviation.

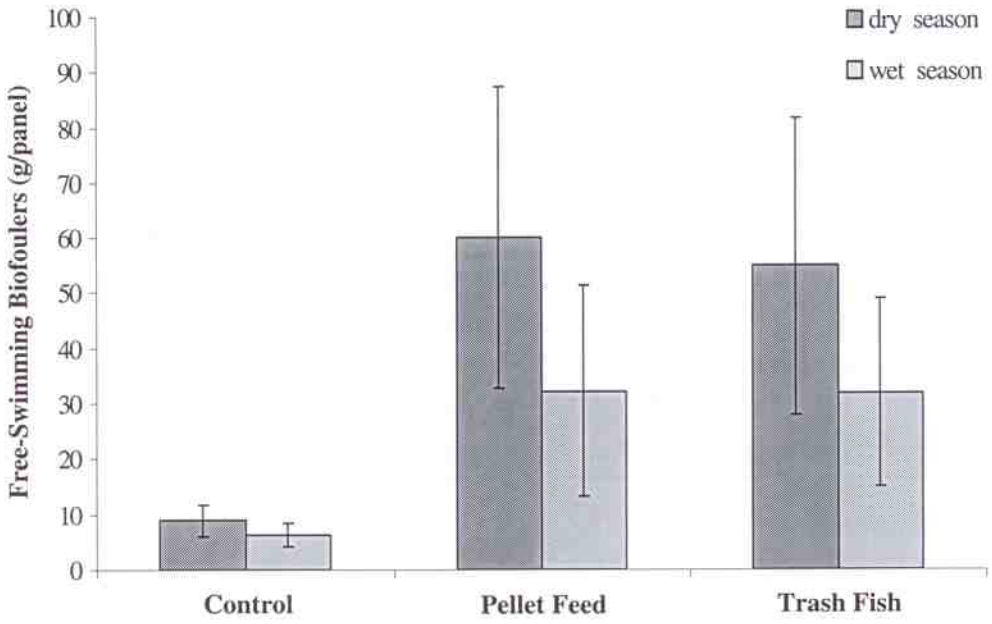


Fig. 7. Effects of fish feed and season on the biomass of and free-swimming biofoulers on experimental net panels at Jaha estuary. Vertical line on bar indicates standard deviation.

The variations in some of the main physical parameters are shown in **Table 1**. During the dry season, the pH, temperature, salinity and the amount of dissolved oxygen of the water were significantly higher (all $p < 0.01$) in Sangga Besar as compared to Jaha for surface and bottom waters. Turbidity readings were however significantly higher ($p < 0.0001$) in Jaha than in Sangga Besar for surface and bottom waters. In Jaha, the pH, salinity and turbidity readings were significantly higher ($p < 0.001$) during the dry season as compared to the wet season, while temperature and dissolved oxygen remained constant.

The environmental conditions in both rivers appeared to be conducive to development of biofouling organisms. Two major environmental factors in the estuary are salinity and substratum. Both have profound influence on the distribution of estuarine organisms. With respect to the present study, while salinity may be suitable to biofouling organisms, the substratum i.e. cage nets, must be able to provide attachment surface as well as a suitable position for the organisms to obtain food and nutrients, and without being significantly affected by other physicochemical factors such as dissolved oxygen, pH, turbidity, toxic (fish) metabolites, pollutants, etc. Since net cages are sited in the surface water, net biofoulers could experience extreme variability in salinity, turbidity and current speed due to the tide and river discharge. The higher salinity, dissolved oxygen and turbidity experienced in Jaha during the dry season appeared to be more conducive than during the wet season for biofouling development, both for attached and free-swimming forms. The average surface salinity during the dry season was about 10 ppt higher than during the wet season, whereas surface turbidity was about 3 times higher. Concomitantly, the biofouling rates increased by a factor of 1.5 - 2 times from wet to dry season, and had reached maximum development in 4 wk as opposed to 12 wk.

Table 1. Mean Values of Some Environmental Parameters Recorded inside the Cages at Jaha and Sangga Besar Estuary during the Dry and Wet Seasons (SD in parentheses).

(a) Jaha (Dry Season)

Parameter	Pellet		Trash Fish		Control	
	Surface	Bottom	Surface	Bottom	Surface	Bottom
pH	7.04 (0.12)	6.82 (0.11)	7.08 (0.11)	6.90 (0.13)	7.05 (0.11)	6.93 (0.15)
Temperature (°C)	29.67 (1.39)	29.68 (1.44)	30.00 (1.38)	29.80 (1.30)	29.62 (1.31)	29.95 (1.54)
Salinity (ppt)	24.84 (4.49)	24.13 (4.36)	24.87 (4.35)	24.01 (4.27)	25.07 (4.49)	24.48 (4.19)
DO (mg l^{-1})	3.74 (1.97)	3.07 (1.48)	3.73 (1.99)	3.27 (1.19)	3.63 (1.97)	3.18 (1.63)
Turbidity (NTU)	13.80 (5.68)	21.00 (7.74)	25.33 (6.02)	34.87 (12.38)	12.67 (7.34)	46.60 (6.00)

(b) Jaha (Wet Season)

Parameter	Pellet		Trash Fish		Control	
	Surface	Bottom	Surface	Bottom	Surface	Bottom
pH	7.00 (0.51)	6.86 (0.44)	7.10 (0.54)	6.88 (0.40)	6.97 (0.53)	6.88 (0.53)
Temperature (°C)	29.35 (0.93)	30.22 (0.91)	29.42 (1.01)	30.25 (1.05)	29.42 (0.89)	30.33 (0.94)
Salinity (ppt)	15.83 (5.99)	18.47 (6.22)	15.57 (6.20)	18.22 (6.20)	16.72 (6.59)	18.15 (7.01)
DO (mg l^{-1})	4.65 (2.66)	3.05 (2.50)	4.44 (2.73)	3.43 (2.71)	4.14 (2.66)	2.75 (2.36)
Turbidity (NTU)	5.57 (3.03)	5.29 (1.35)	4.52 (1.50)	5.14 (1.90)	5.48 (1.63)	7.90 (2.47)

(c) Sangga Besar (Dry Season)

Parameter	Pellet		Trash Fish		Control	
	Surface	Bottom	Surface	Bottom	Surface	Bottom
pH	7.19 (0.20)	7.14 (0.19)	7.25 (0.19)	7.17 (0.16)	7.17 (0.17)	7.13 (0.16)
Temperature (°C)	30.08 (0.34)	30.61 (0.55)	30.15 (0.45)	30.70 (0.50)	29.77 (0.43)	30.62 (0.41)
Salinity (ppt)	28.18 (2.94)	28.78 (2.91)	28.31 (3.14)	28.77 (2.91)	28.03 (3.22)	28.82 (3.01)
DO (mg l^{-1})	4.06 (1.42)	3.23 (1.07)	4.05 (1.59)	3.65 (1.69)	4.09 (1.52)	3.21 (0.80)
Turbidity (NTU)	16.93 (5.87)	21.46 (5.33)	5.4 (0.73)	9 (1.73)	15.6 (6.11)	21.06 (5.62)

Low salinity could have a depressive effect on biofouling since the causative organisms are largely marine species comprising amphipods, copepods, polychaetes and cirripedes as the major free-swimming biofoulers, while *Glacilaria*, bryozoans, cnidarians and barnacles are the common attached biofouling species. The reduction in natural food availability during the wet season could also limit the mean biomass of biofouling. In a concurrent study of the water column nutrients and chlorophyll-*a*, Wong (in preparation) found significantly higher

mean nitrate (3.91 μmol l^{-1}) and chlorophyll-*a* (49.6 μgl^{-1}) concentrations during the dry season (lower salinity) as compared to the wet season (3.03 μmol^{-1} and 17.3 $\mu g\ l^{-1}$, respectively). The dissolved forms and ratios of nitrogen and phosphorus (6 : 1) released from fish farms promote phytoplankton growth (Silvert and Sowles, 1996). Marine phytoplankters are most often limited in their growth by a shortage of nitrogen (as nitrate or ammonium) rather than phosphate (Tett, 2002).

The effects of feed treatment on chlorophyll-*a* concentrations (investigated only in Jaha) were as follows: 49.3253 μgl^{-1} (pellet) = 43.4810 μgl^{-1} (trash fish) > 31.9080 μgl^{-1} (control), respectively (**Fig. 8**) ($\alpha = 0.05$). It appears that both types of feed released significant amount of inorganic nutrients that were utilized by phytoplankton which were in turn consumed by the biofoulers. Chong *et al.* (this proceedings) found significantly higher concentrations of inorganic (particularly phosphates and ammonia) and particulate nutrients at cage sites as compared to non-cage sites.

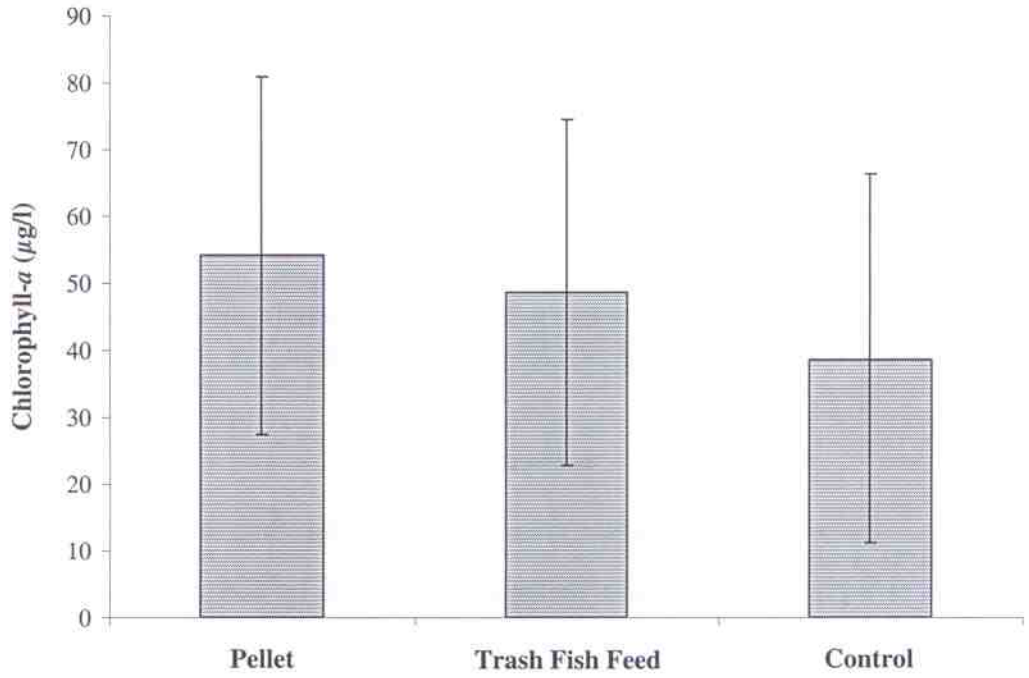


Fig. 8. Mean chlorophyll-*a* concentrations recorded inside fish cages (pellet and trash fish) and control (outside, no feed) in Jaha estuary during the dry season. Vertical line on bar indicates standard deviation.

The slightly higher biofouling (especially by attached forms) in the "pellet" cages as compared to "trash fish" cages in both rivers and seasons could be due to higher particulate release from the formulated pellets. The pellet feed had a high percentage of fine unbound feed powder. Release of these fine particles probably encouraged the rapid growth of suspension or filter feeders such as barnacles and bryozoans.

Conclusion

Feeding cage fish with formulated feed pellets and mashed trash fish equally contributes to the development of biofouling organisms on cage nets. The experimental results show that during the dry season biofouling increased rapidly and reached near maximum (wet) biomass at the fourth week for both attached and free-swimming forms, respectively. The mean biomass of attached biofoulers was significantly higher in Sangga Besar estuary (351.5 g) than in Jaha estuary (307.9 g), representing high and low density cage culture respectively. There was however no significant difference in free-swimming biofouling rates between estuaries. For both estuaries, feed type had the following effects on biofouling rates: Pellet > Trash feed > Control, irrespective of the type of biofoulers. During the wet season net biofouling reached maximum biomass only after 11 weeks. The mean biofouling rates of both forms were however significantly higher (1.5 - 2.0 times) during the dry than wet season. The development of cage net biofouling organisms in both rivers and seasons are likely modified by variations in environmental parameters, particularly salinity.

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APPENDIX 2

Development and Short-Term Dynamics of Macrofouling Assemblages on Fish-Cage Nettings in a Tropical Estuary

John Madin, V.C. Chong & Badrulnizam Basri (2009). Development and short-term dynamics of macrofouling assemblages on fish-cage nettings in a tropical estuary. Estuarine, Coastal and Shelf Science 83: 19 – 29.

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Development and short-term dynamics of macrofouling assemblages on fish-cage nettings in a tropical estuary

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ABSTRACT

A study was conducted at a fish culture farm in the Jaha River estuary, Malaysia, to examine the structure and development of macrofouling assemblages on floating net-cages. The study was conducted during the dry (August–October 2001) and wet (December–February 2002) seasons. Biofouling on 1.6 cm mesh net panels (size 0.2 m × 2 m) suspended inside (P, T) and outside (O) experimental net-cages was monitored every week until net openings were completely occluded by macrofouling organisms (8 wk and 12 wk for dry and wet seasons respectively). Seven species (6 phyla) of sessile organisms and 23 species (3 phyla) of non-sessile associates were recorded. Macro-colonization of net panels began with the hydroid *Plumularia* sp. irrespective of season and treatment (P, T, and O), while other species only appeared after 1 or 2 weeks of immersion. Inside net-cages where water flow was slow (mean < 6 cm s^{-1} at 0.50–0.75 m depth); macroalgae (*Polysiphonia* sp.), anthozoans (unidentified anemone), barnacles (*Balanus amphitrite*), amphipods (*Gammaropsis* sp. & *Photis* sp.), and tanaids (*Leptognathia* sp.) were dominant on the net panels during the dry season. In the wet season, hydroid (*Plumularia* sp.), mussel (*Xenostrobus mangle*), and nematode abundance were however significant. With stronger water flow (mean ≈ 20 cm s^{-1}) as occurring outside the net-cages, macrofouling assemblages for both seasons comprised mainly *Plumularia* sp. and *Gammaropsis* sp. The macrofouling assemblage showed a clear succession of species that occupied different layers of the net panels. The study shows that while organic enrichment and retarded water flow together enhance the development of macrofouling assemblages, salinity, depth, substrate (net) area and species competition specifically influence community structure, colonization, and depth distribution of the macrofouling organisms.

1. Introduction

Macrofouling assemblages have been studied for their succession, settlement, competition, recruitment and stability (e.g. Kay and Butler, 1983; Anderson and Underwood, 1994; Butler and Connolly, 1996). An important purpose of studying the biofouling process is to address the problem of its prevention or control (see Abarzua and Jakubowski, 1995) which has been estimated to incur an annual expenditure of over US$6 billion by the global shipping industry alone as a result of higher fuel consumption and regular cleaning maintenance (Adkins et al., 1996; Callow and Callow, 2002). The biofouling problem also affects the oil and gas industries, fishing and aquaculture equipment, cooling systems of power plants as well as living aquatic organisms. In aquaculture, biofouling eventually occludes the openings of pen- or cage-nets causing serious oxygen and water quality problems which if not addressed could result in fish kills (Aarsnes et al., 1990; Loland, 1993). The frequent cleaning of nets is not only costly and labor-intensive but often gives rise to loss of stocked fish due to net changes and damage, and disturbance of feeding regimes causing lower growth rates of the cultured fish (Hodson et al., 1995, 1997). Uncleaned nets on the other hand can cause severe physical stress on the cage nettings during strong current flow when they could tear (Phillippi et al., 2001; Swift et al., 2006).

Marine macrofouling occurs as encrustations of algae, cnidarians, barnacles, bryozoans, tubeworms and mussels. The colonization of artificial surfaces is thought to be initiated or encouraged by microfouling organisms such as bacteria, diatoms and fungi that form the primary organic film or biofilm (Wahl, 1989; Corner et al., 2007). In general, biofouling assemblages are characterized by continuous changes in species composition in response to the biotic and abiotic factors over time (Greene and Schoener, 1982; Connell, 2001).

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Although biofouling of artificial substrates has been well studied, less studied is biofouling pertaining to the aquaculture environment where fish pens or cages release and increase nutrient and organic inputs, and act as current-reducing structures thought to enhance biofouling (Ruokolahti, 1988; Huang et al., 1999; Qian et al., 2001). Even less studied is the problem of biofouling on fish-cage nettings in tropical marine waters where cage culture is now commonly practiced and becoming the fastest growing mariculture industry in many countries (Beveridge and Little, 2002; Subasinghe, 2004). The use of most commercially available, anti-fouling chemicals or coatings on cage nettings is largely restricted due to concern of environmental toxicity as well as consumer preference that may jeopardize the market image of cultured fish (Wu, 1995; Hodson et al., 1997; Champ, 2000; Braithwaite et al., 2007). For these reasons, it is imperative that the natural control of biofouling or environment-friendly methods be used. Such methods require a better understanding of the fouling community of cage nettings particularly how it interacts with the physical environment and aquaculture itself. As pointed out by Braithwaite and McEvoy (2005), even though research on biofouling of fish nettings had begun 30 years ago, quantitative data is relatively patchy or of limited use. Thus, the objective of the present study was to study the community structure and short-term dynamics of macrofouling assemblages on fish-cage nettings, in relation to fish culture, season (wet or dry) and period of immersion. We examine species composition, abundance and colonization of macrofouling organisms on suspended fish nettings used in floating fish cages deployed in coastal bays and estuaries.

2. Materials and methods

2.1. Study site

The study was carried out in a fish culture farm located in the Jaha River estuary within the Matang Mangrove Forest Reserve (MMFR), Malaysia (Fig. 1). The main estuaries in the MMFR are used for culturing the giant sea perch (*Lates calcarifer*), golden snapper (*Lutjanus johnii*), and red snapper (*Lutjanus argentimaculatus*) in floating net-cages. Fish culture farms are particularly dense (approximately 4000 net-cage units) in the estuary of Sangga Besar River. However in the Jaha River estuary, there are only two farms with a total of approximately 600 floating net-cages. The Jaha estuary is shallow averaging 3 m in depth. Water is well mixed and the tides are semi-diurnal, with a tidal amplitude of 2.5 m. Rain is experienced throughout the year, but there are two peaks of heavy rainfall coinciding with the onset of the southwest and northeast monsoons in May and November respectively.

2.2. Experimental design and layout

Two experiments were conducted, one during the 'dry season' (August–October 2000) and the other during the 'wet season' (December 2000–February 2001). Six non-fouled net-cages with 1.6 cm mesh size for stocking fingerlings were set up for each experiment in a fish culture farm containing 258 net-cage units. Each experimental net-cage measured 2.5 m $\times$ 2.5 m in surface area, with a depth of 1.5 m. The net-cages were set up in triplicates to receive either ground trash-fish (T1, T2, and T3) or pellet feed (P1, P2, and P3). To ensure that all experimental net-cages were exposed to the same or near similar current regime, net-cages were positioned at the downstream end of the farm (Fig. 2a). This position reduced cross-contamination from the downstream during fish feeding which was carried out at low slack tide, although feed given were completely consumed by fish within the first 15 min. The experimental net-cages were set up within the fingerlings' rearing area of the fish culture farm where high macrofouling rates occurred due to the use of small mesh size net-cages (see Fig. 2a). The farm size limitation only allowed the triplicates to be linearly arranged one after another (triplets) along the long axis of the river. Nevertheless, each member of a treatment triplet (e.g. T1) was assumed to share similar physical conditions as their counterpart (P1) on the other treatment triplet. Preliminary current measurements inside similarly arranged net-cages had shown that the flow rates were not significantly different among members of a triplet (see also Results). The better arrangement of each outer cage on the flood side of the cage block was not possible due to farm size limitation. Any extension of treatment cages over to the adult cage block would mean the treatment cages will be subject to different flow hydrodynamics during ebb flow since the net mesh size of adult cages was much larger.

Each experimental net-cage was initially stocked with 200 giant sea perch (*Lates calcarifer*) fingerlings (mean wet weight of 27 $\pm$ 4.5 g) and daily feed rate was 3–4% of the total biomass of stocked fish. The home-made pellets were rod shaped and made from poultry offal meal at a mean size of 2 mm diameter. Trash-fish were ground to a sticky pulp containing particulates of very variable sizes. The use of ground trash-fish is the common practice of fish feeding.

2.3. Experimental method and sampling

Green multifilament nylon net panels each of 0.2 m $\times$ 2 m dimensions and of 1.6 cm mesh size (2.3 cm stretched mesh size) were placed inside each experimental net-cage (Fig. 2b). Net multifilaments had a mean diameter of 1.2 mm. The lower end of each net panel was weighed down using lead sinkers to a depth of 2 m below the water surface, while its upper end was tied to an aluminium bar held horizontally across the net-cage frame (Fig. 2c). The upper 0–1.32 m of the net panels was vertically positioned while the lower part (1.32–2 m) bent over the net-cage bottom. Three sets of net panels "without caging" (O1, O2, and O3) were placed just outside the net-cages, approximately 5 m distance from treatments P and T (see Fig. 2a). Thus the macrofouling assemblages at O represented organisms without the effects of fish rearing (fish feed, nutrient and faecal material) and impeded water flow as compared to treatments P and T which had all the above effects. Macrofouling development on the pre-weighed net panels was then monitored each week by gently removing one panel from each experimental net-cage with the aid of a dip net (2.5 mm mesh size), until the completion of the experiment. The experiments were terminated at the end of the 8th week for the dry season and the 12th week for the wet season, that is, when the net meshes were completely occluded by macrofouling. Removed net panels with fouling organisms were immediately preserved in buffered 10% formalin, in separate 1-l plastic jars.

2.4. Physical and chemical parameters

Salinity, turbidity, temperature, pH and dissolved oxygen (DO) were measured fortnightly at the surface (0.5–0.75 m depth) and net-cage bottom (1–1.5 m depth), using a YSI 3800 multi-parameter sonde. The water parameters were recorded 2 h before fish feeding, and before the experimental net panels were sampled. Water velocity at the experimental net-cages was also measured fortnightly at 0.5–0.75 m depth by a Toho Dentan electric current meter (Model CM-2) during flood flow (beginning with neap tide), 1–2 h after low slack tide.

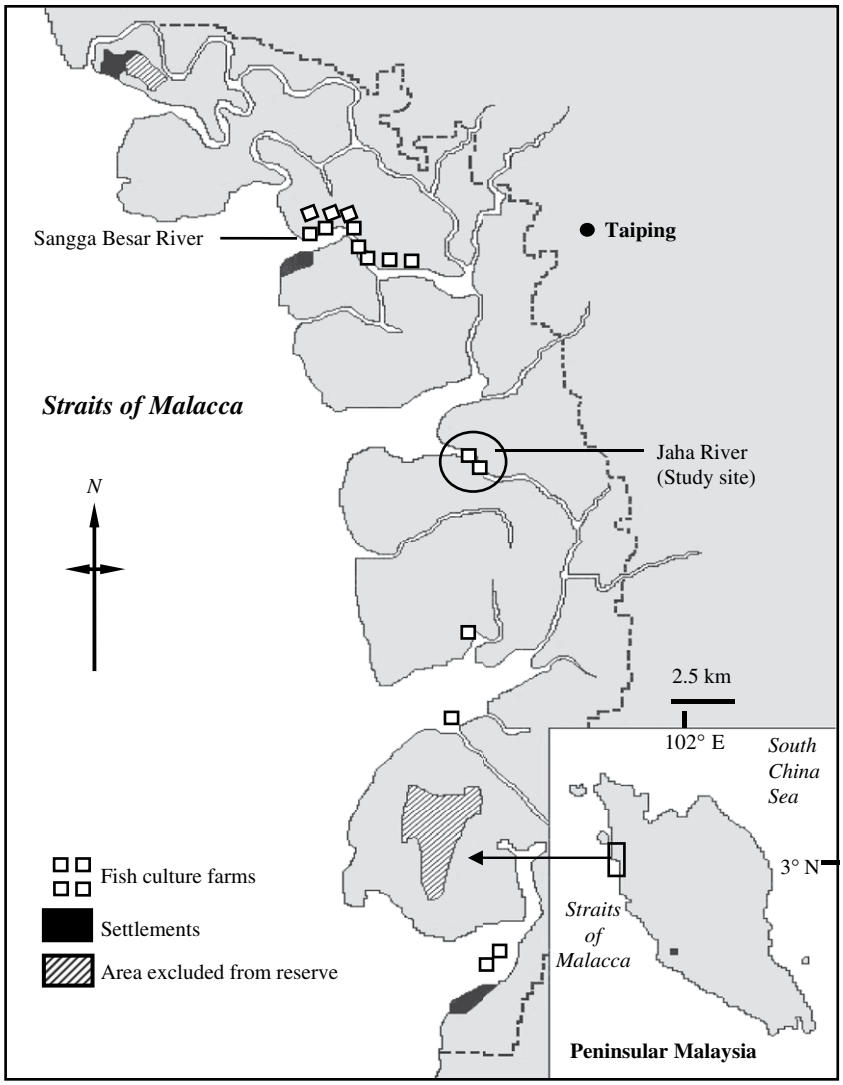

Fig. 1. Location of study site at Jaha River estuary in the Matang Mangrove Forest Reserve (MMFR), Perak, Malaysia.

2.5. Laboratory treatment and analysis

In the laboratory, each net panel was gently agitated and removed from its jar. The agitation was to remove non-sessile organisms for later examination. The difference in weight of net panel before and after the experiment represented the weight of the sessile biofoulers (g per 0.4 m^2). To further quantify the abundance of sessile organisms, the net panel was equally subsampled from three depth strata, upper (0–0.66 m), middle (0.66–1.32 m), and bottom (1.32–2 m) (see Fig. 2b). To ensure random sampling, a random numbers table was used to pick a coordinate pair read as the node or intersection of the net grid (meshes). Three subpanels of 5 cm × 5 cm area were randomly picked and cut out from each strata of the net panel (Fig. 2d). Stratified random sampling was done to ensure equal samplings at different depth layers since certain species appeared to be preferentially distributed.

Sessile organisms were quantified based on cover (%). Cover was determined by estimating the area occupied by the species under a Leica MZ8 microscope. This procedure was carried out by simply estimating the occupied surface area of each net filament (inter-knot distance = 12.5 mm), and finally summing up the total number of filaments surface area occupied by the species. A fully occupied filament would give a score of one filament surface area occupied. The total number of filaments per 25 cm^2 subpanel was 40 (see Fig. 2d).

Non-sessile associates or mobile macrofauna, which had dropped to the bottom of the bottle after agitation, were collected by sieving the entire fluid through a 125 μm mesh Endecott sieve and quickly washed with running tap water to remove fine sediments. They were then placed onto a pre-weighed wire gauze of the same mesh size, blot-dried, weighed together and immediately resuspended in 70% alcohol solution. A Stempel pipette (0.5 ml) was used to obtain a homogeneous subsample which was placed in a Petri dish and observed under a stereo microscope or if necessary a compound microscope. Subsamplings were taken and species abundance was enumerated until no new species were encountered. Usually between 10 and 15 subsamplings were done. Unlike sessile organisms, the abundance of mobile macrofauna could not be defined by depth zonation but for the entire net panel.

2.6. Computation and statistical analysis

The percentage cover of the sessile organism was estimated as follows:

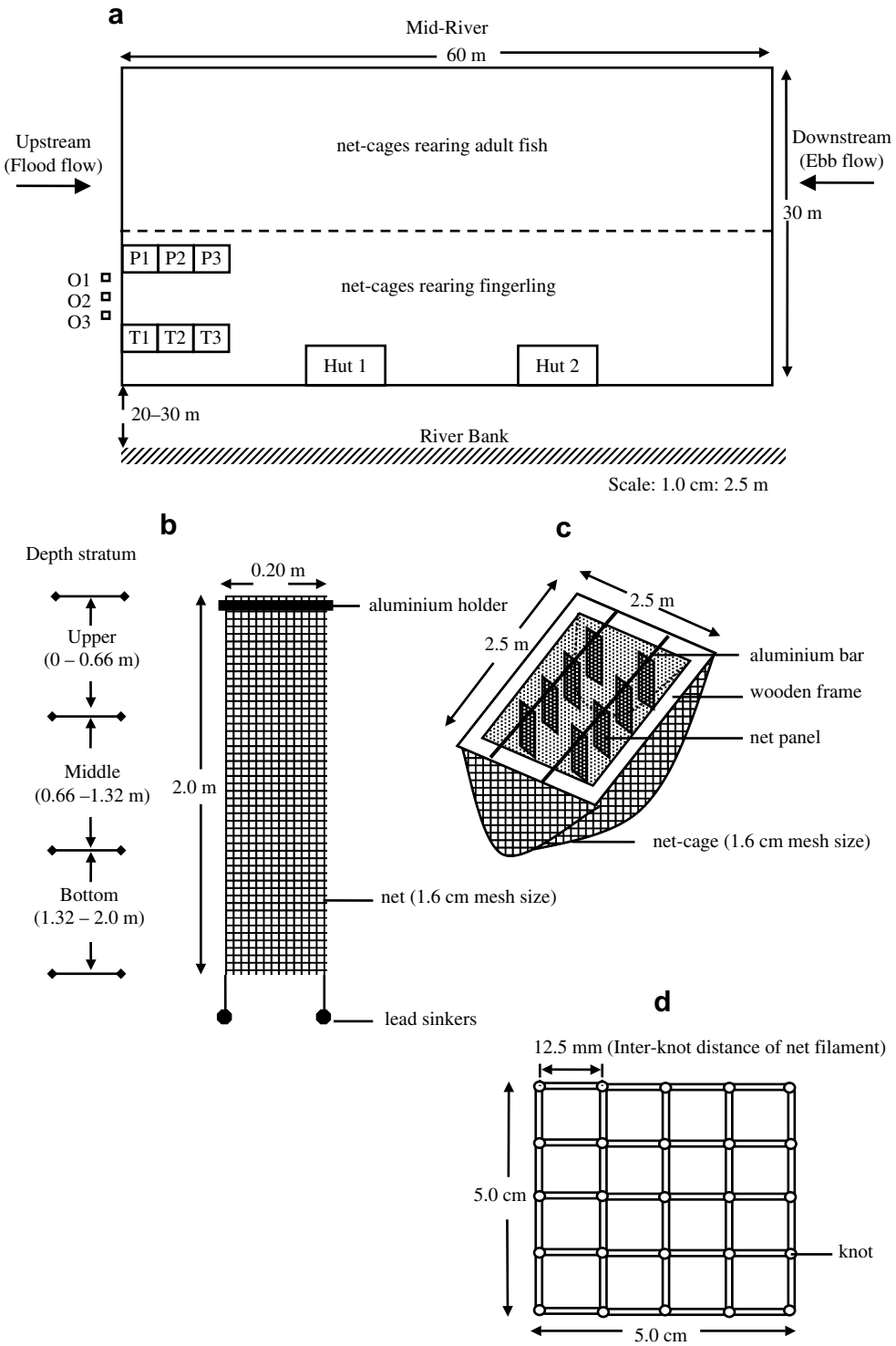

Fig. 2. (a) Layout of experimental floating net-cages in fish culture farm (size 60 m × 30 m, 258 net-cages) in Jaha River estuary. Net panels were hung inside the net-cages with cultured fish given trash-fish feed (T1, T2 and T3) and home-made pellet feed (P1, P2, P3), and outside the net-cages, without cultured fish and feed (O1, O2, O3); (b) Diagram of single experimental net panel showing sampled depth strata; (c) Diagram showing how several net panels were positioned inside an experimental net-cage; (d) Diagram of sampled subpanel of 5 cm × 5 cm comprising 40 filaments each.

$$\text{Percentage cover}(\%) = \frac{\text{total number of filament lengths occupied}}{40} \times 100$$

[Given that total number of filaments per 5 cm × 5 cm of the subpanel was 40, see Fig. 2d]

The density of non-sessile associates (no. per 100 cm^2 or 1 dm^2 of net panel) was estimated as follows:

$$\text{Density} = \frac{\text{Mean number of enumerated individuals}}{0.5} \times \frac{\text{Volume of sample fluid(ml)}}{40}$$

[Given that the Stempel pipette sampled 0.5 ml and the total area of net panel was 40 dm^2].

Computed percentage cover and density data were subjected to arcsine and logarithmic [$\log_{10}(x+1)$] transformations, respectively, so as to achieve normality and homogeneity of variance before statistical analysis (Zar, 1998).

A 3-factor ANOVA was carried out to investigate the effects of treatment (T, P, and O), season (dry, wet) and immersion time (week 1, 2, 3, …, 12) on the physical factors and biomass, percentage cover and density of sessile and non-sessile macrofoulers. The Student–Newman–Keuls test was used for multiple comparisons of the means. Both tests were performed using Statistica Ver. 8 software.

Principal components analysis (PCA) was carried out to study the net panel colonization by the macrofouling community as possibly influenced by the type of fish feed given and immersion time during the dry and wet seasons. Orloci's chord distances were computed instead of Euclidean distances so as to avoid the paradox problem associated with the latter when species abundance data are used (Legendre and Gallagher, 2001). Chord distances were computed from the species abundance data via a transformation program downloaded from http://www.bio.umontreal.ca/casgrain/en/labo/transformations.html. The program converts a matrix of species abundance in such a way that the Euclidean distance among rows of the transformed matrix is equal to the chord distance among rows of the original data matrix. PCA of the species abundance data was performed using the CANOCO ver. 4.02 software (ter Braak and Smilauer, 1998). A correlation biplot on the first two principal components axes was obtained.

3. Results

3.1. Environmental conditions

Salinity and turbidity readings for both surface and bottom were statistically higher ($P < 0.001$) during the dry season compared to the wet season (Table 1). Temperature, dissolved oxygen, and pH between wet and dry seasons were not significantly different ($P > 0.05$). Surface salinity during the dry season averaged 24.9 in comparison to 16 recorded during the wet season. In the wet season, surface and bottom salinities could differ by as much as 3 over a depth of 3 m (see Table 1), but this difference may become more pronounced during episodic squally showers when surface salinity of 5 had been recorded. Water flow velocities measured outside the net-cages (O) were significantly higher ($P < 0.05$) than inside net-cages (P and T), but not among net-cages (P and T) for both dry and wet seasons ($P > 0.05$) (Fig. 3). There was a significant attenuation of current velocity by 80–90% at P1 and T1 as the current first encountered the net-cages, and attenuation increased only slightly to a maximum of 96% farther to the leeside (at P2, P3, T2, T3). Only with clean unfouled nets (Week 0) was the attenuated velocity at P1 (T1) significantly higher than at P2 (T2) and P3 (T3) ($P < 0.05$).

3.2. Species composition

The macrofouling assemblages on the net panels comprised of 7 species of sessile organisms from 6 phyla, and 23 species of non-sessile associates from 3 phyla (Table 2). Among the sessile organisms found on the net panels were macroalgae (*Polysiphonia* sp. and *Enteromorpha clathrata*), unidentified sea anemone (Anthozoa), hydroids (*Plumularia* sp.), mussels (*Xenostrobus mangle*), and barnacles (*Balanus amphitrite*). Composition of sessile organisms for both dry and wet seasons was very similar. *Plumularia* sp. and *Polysiphonia* sp. were the most dominant sessile organisms. Almost similar species of sessile organisms were found for the three different treatments (T, P, and O).

The non-sessile organisms with the sessile community on the net panels belonged mostly to the phyla Arthropoda, Annelida and Nematoda. The Arthropoda were represented by three crustacean orders, mostly gammaridean Amphipoda, Copepoda and Tanaidacea. There were 5 amphipod species from the families of Isaeidae (*Gammaropsis* sp., *Photis* sp. and *Cheirophotis* sp.), Corophiidae (*Corophium* sp.), and Amphilochidae (*Gitanopsis* sp.). Almost 90% of the whole population of non-sessile macrofauna was amphipods from the family Isaeidae (*Gammaropsis* sp. and *Photis* sp.).

3.3. Species colonization

The first two axes derived from PCA of the species abundance data explained 42% of the total variance in the species abundance data. The PCA biplots illustrate two courses of net panel colonization by sessile organisms inside the net-cages, based on the community structure which was determined by season and period of immersion (top and bottom left quadrants, Fig. 4). For both seasons, the initial colonizer at all depths was the colonial hydroid, *Plumularia* sp. However, in the dry season, as colonization proceeded to the 3rd or 4th week, the hydroid was replaced by the macroalgae *Polysiphonia* sp. and *Enteromorpha clathrata* at the top

Table 1
Mean values (mean ± SD) of environmental parameters recorded during the dry (August–October 2000) and wet (December 2000–February 2001) seasons, in the floating net-cage farm at Jaha River estuary. Measurements were taken fortnightly for two and three months during dry and wet seasons respectively.

	Inside net-cages Trash-fish feed (T)		Inside net-cages Pellet feed (P)		Outside net-cages No feed (O)	
	Surface	Bottom	Surface	Bottom	Surface	Bottom
Dry season						
pH	7.08 ± 0.11	6.90 ± 0.13	7.04 ± 0.12	6.82 ± 0.11	7.05 ± 0.11	6.93 ± 0.15
Temperature (°C)	30.00 ± 1.38	29.80 ± 1.30	29.67 ± 1.39	29.68 ± 1.44	29.62 ± 1.31	29.95 ± 1.54
Salinity	24.87 ± 4.35*	24.01 ± 4.27*	24.84 ± 4.49*	24.13 ± 4.36*	25.07 ± 4.49*	24.48 ± 4.19*
DO ($mg\,l^{-1}$)	3.73 ± 1.99	3.27 ± 1.19	3.74 ± 1.97	3.07 ± 1.48	3.63 ± 1.97	3.18 ± 1.63
Turbidity (NTU)	25.33 ± 6.02*	34.87 ± 12.38*	13.80 ± 5.68*	21.00 ± 7.74*	12.67 ± 7.34*	46.60 ± 6.00*
Flow velocity ($cm\,s^{-1}$)	4.47 ± 3.87		3.80 ± 1.00		20.00 ± 1.60†	
Wet season						
pH	7.10 ± 0.54	6.88 ± 0.40	7.00 ± 0.51	6.86 ± 0.44	6.97 ± 0.53	6.88 ± 0.53
Temperature (°C)	29.42 ± 1.01	30.25 ± 1.05	29.35 ± 0.93	30.22 ± 0.91	29.42 ± 0.89	30.33 ± 0.94
Salinity	15.57 ± 6.20	18.22 ± 6.20	15.83 ± 5.99	18.47 ± 6.22	16.72 ± 6.59	18.15 ± 7.01
DO ($mg\,l^{-1}$)	4.44 ± 2.73	3.43 ± 2.71	4.65 ± 2.66	3.05 ± 2.50	4.14 ± 2.66	2.75 ± 2.36
Turbidity (NTU)	4.52 ± 1.50	5.14 ± 1.90	5.57 ± 3.03	5.29 ± 1.35	5.48 ± 1.63	7.90 ± 2.47
Flow velocity ($cm\,s^{-1}$)	5.14 ± 4.47		5.80 ± 5.00		20.80 ± 4.80†	

† Indicates significant difference ($P < 0.05$) amongst treatment (T, P, O).
* Indicates significant difference ($P < 0.05$) between seasons (dry, wet).

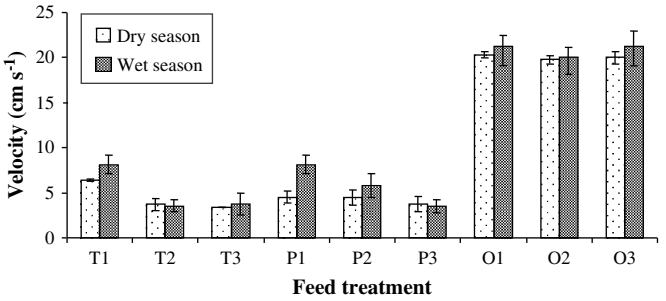

Fig. 3. Mean water flow velocity (cm s^{-1}) recorded during neap tide at T1, T2 and T3 (cages with trash-fish feed), P1, P2, and P3 (cages with pellet feed), and O1, O2 and O3 (outside the net-cages), during dry and wet seasons. Measurements were taken fortnightly for two and three months during dry and wet seasons respectively.

stratum, and by anthozoans and barnacles at both the middle and bottom strata of the net panels. On the other hand, the development of macrofouling assemblages in the wet season appeared much slower, and the hydroid was displaced by yet another, increasingly dominant species, *Xenostrobus mangle*, particularly at the upper stratum, after 6 weeks of submersion.

Inside the net-cages, the trends of net panels colonization by macrofouling organisms were rather similar for both type of fish feed (pellet or trash-fish feed) irrespective of the seasons. Outside the net-cages, the type of macrofouling species present did not differ with season, or the period of immersion (top right quadrant). These macrofoulers comprised of mainly the same hydroids and associated non-sessile organisms.

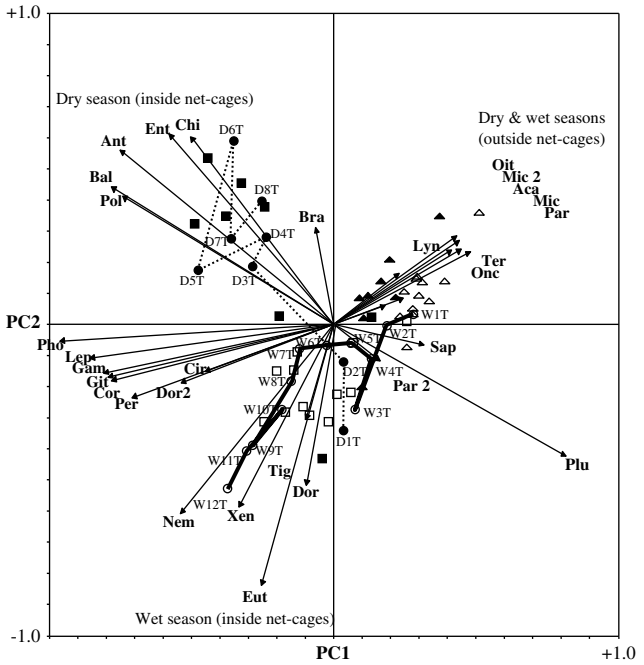

Fig. 4. PCA biplots of the abundance of net panel macrofouling organisms (arrows) on experimental net panels (squares, circles and triangles) for dry and wet seasons. Solid and dash lines linking filled or open circles trace the weekly (numerals) macrofouling progression in net-cages given trash-fish feed (T) during wet (W) and dry (D) seasons respectively. Types of treatment: pellet feed in dry season (■); pellet feed in wet season (□); trash-fish feed in dry season (●); trash-fish feed in wet season (○); outside net-cages in dry season (▲); outside net-cages in wet season (△). Full taxa names are given in Table 2.

Table 2
List of sessile and non-sessile macrofouling organisms on net panels placed inside net-cages with trash-fish feed (T), inside net-cages with pellet feed (P), and outside net-cages without fish and feed (O), during the dry and wet seasons in a fish culture farm at Jaha River estuary. Acronyms are given in parentheses. + indicates occurrence.

Group	Taxa	Dry season			Wet season		
		T	P	O	T	P	O
Algae	*Polysiphonia* sp. (Pol)	+	+	+	+	+	+
	Enteromorpha clathrata (Ent)	+	+	+	+	+	+
	Lyngbya sp. (Lyn)	+	+	+	+	+	
Bivalves	*Xenostrobus mangle* (Xen)	+	+	+	+	+	+
Anthozoans	Undetermined species (Ant)	+	+	+	+	+	+
Hydroids	*Plumularia* sp. (Plu)	+	+	+	+	+	+
Barnacles	*Balanus amphitrite* (Bal)	+	+	+	+	+	
Amphipods	*Gammaropsis* sp. (Gam)	+	+	+	+	+	+
	Photis sp. (Pho)	+	+	+	+	+	+
	Cheirophotis sp. (Chi)	+	+	+		+	+
	Corophium sp. (Cor)	+	+	+	+	+	+
	Gitanopsis sp. (Git)	+	+	+	+	+	+
Tanaids	*Leptognathia* sp. (Lep)	+	+	+	+	+	+
Isopod	*Cirolana* sp. (Cir)	+	+	+	+	+	+
Decapods	Brachyura megalopa (Bra)	+	+	+		+	+
Copepods	*Euterpina acutifrons* (Eut)	+	+	+	+	+	+
	Tigriopus sp. (Tig)	+	+	+	+	+	+
	Oncaea sp. (Onc)	+	+	+	+	+	+
	Saphierella-like copepodid (Sap)	+			+	+	+
	Acartia pacifica (Aca)	+	+	+	+	+	+
	Microcalanus sp. 1 (Mic)	+	+	+	+	+	+
	Microcalanus sp. 2 (Mic 2)			+	+	+	+
	Paracalanus sp. 1 (Par)		+	+	+	+	+
	Paracalanus sp. 2 (Par 2)	+		+	+	+	+
	Oithona simplex (Oit)	+	+	+	+		+
Annelida	*Perinereis* sp. (Per)	+	+	+	+	+	+
	Dorvilleidae sp. 1 (Dor)	+	+	+	+	+	+
	Dorvilleidae sp. 2 (Dor 2)	+	+	+	+	+	+
	Terebellidae sp. (Ter)		+	+	+	+	+
Nematoda	Undetermined species (Nem)	+	+	+	+	+	+

The net-cages, the type of macrofouling species present did not differ with season, or the period of immersion (top right quadrant). These macrofoulers comprised of mainly the same hydroids and associated non-sessile organisms.

Among the non-sessile species that were closely associated with the sessile organisms on net panels placed inside the net-cages were several species of amphipods. These amphipods together with a few other species form the non-sessile assemblage that was observed in both the dry and wet seasons (see Fig. 4). Only *Cheirophotis* sp. and *Gammaropsis* sp. appeared to be associated with the dry season.

There appeared to be a succession of amphipods species on net panels placed inside the net-cages given pellet or trash-fish feed where the dominance of *Gammaropsis* sp. was replaced by *Photis* sp. at the 5th week during the dry season but at the 9th week during the wet season. However, outside the net-cages *Gammaropsis* sp. was dominant throughout the dry and wet season studies.

3.4. Depth distribution of sessile organisms

3.4.1. Dry season

Inside the net-cages and irrespective of the type of fish feed given, *Plumularia* sp. rapidly developed covering the entire net panels within the 1st week, but the species eventually gave way to other macrofouling species which started to appear thereafter and competed with it (Fig. 5a and b). By the 3rd week, *Plumularia* sp. at the surface stratum was reduced to 10% cover, while the two macroalgal species, *Polysiphonia* sp., and *Enteromorpha clathrata*, increased to 40% and 35% cover, respectively. At the surface, *Polysiphonia* sp. dominated throughout the remaining period, achieving maximum cover of 80% at the 8th week. *E. clathrata* however gradually decreased and disappeared by the 8th week (see Fig. 5a).

Anthozoans and the barnacles, *Balanus amphitrite* only conspicuously appeared after 2 or 3 weeks of immersion inside the net-cages; they consistently dominated the middle and bottom strata of the net panels. Anthozoans cover could reach 80% by the 6th week (see Fig. 5a).

The colonization trend of *Plumularia* sp., *Polysiphonia* sp., *Enteromorpha clathrata*, anthozoans, *Balanus amphitrite* and *Xenostrobus mangle* at all depth strata inside the pellet given net-cages was quite similar to that observed in trash-fish given net-cages.

Outside the net-cages, *Plumularia* sp. completely covered the net panels within the 1st week of immersion (Fig. 5c), but its cover on the upper stratum was gradually reduced from the 2nd week due to competition from *Lyngbya* sp., anthozoans and *Xenostrobus mangle*. *Lyngbya* sp. persisted throughout the study period. *Plumularia* sp. persisted longer in the middle and bottom strata due to less aggressive colonization by other sessile species.

3.4.2. Wet season

After 8 weeks of macrofouling in trash-fish given net-cages, the percentage covers of *Polysiphonia* sp. and *Enteromorpha clathrata* on the upper stratum of net panels were found to be significantly lower in the wet season as compared to the dry season ($P < 0.001$). There were almost no colonies of these species at the middle or bottom stratum during the wet season. Anthozoans and barnacles which dominated below the upper stratum were also significantly ($P < 0.05$) lower in cover. On the other hand, the small mussel, *Xenostrobus mangle*, and *Plumularia* sp. had significantly ($P < 0.05$) higher covers during the wet season, on the upper stratum and all strata, respectively.

In pellet given net-cages, the percentage covers of *Polysiphonia* sp., *Plumularia* sp., anthozoans, *Balanus amphitrite*, and *Enteromorpha clathrata* were quite similar ($P > 0.05$) to that in trash-fish given net-cages, except that *Xenostrobus mangle* had significantly

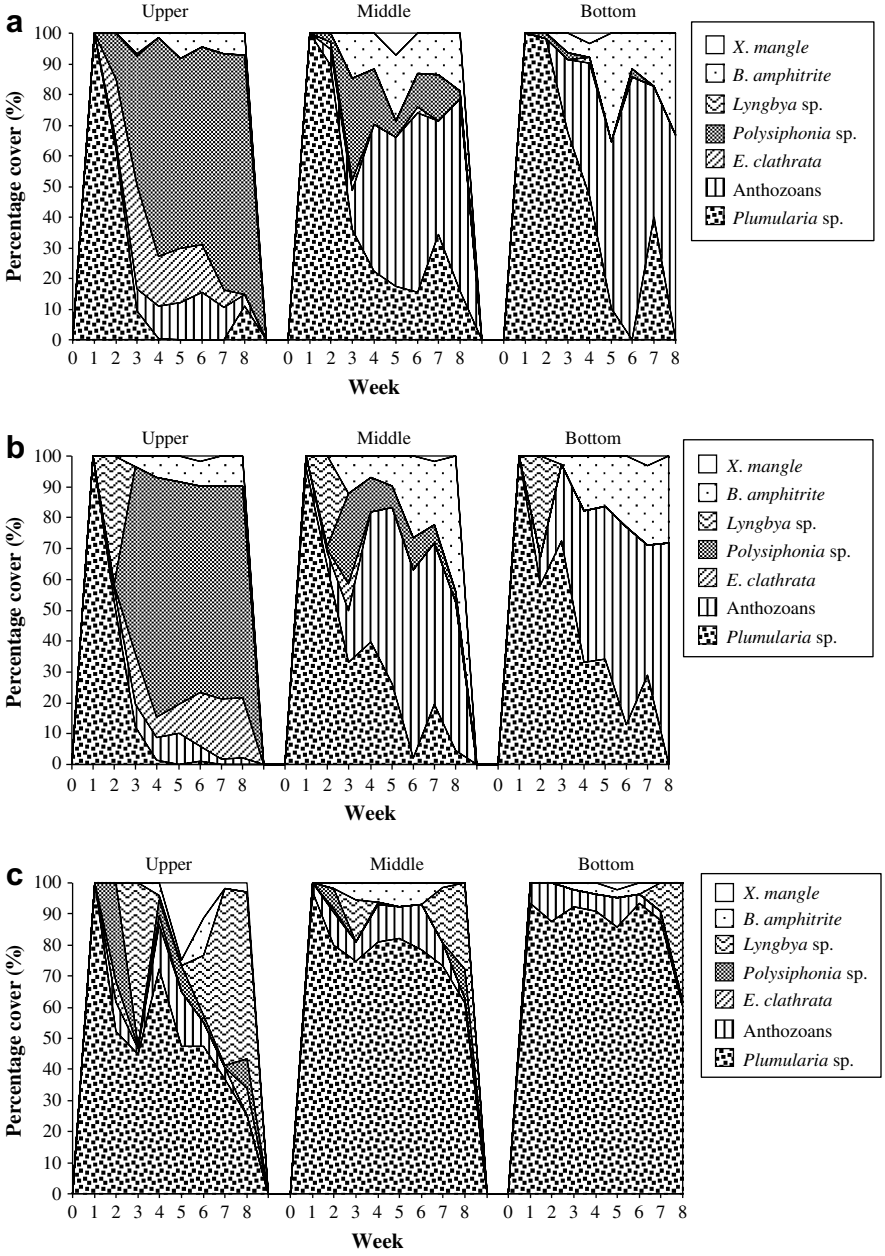

Fig. 5. Temporal changes in percentage cover of sessile macrofouling organisms at the upper, middle and bottom strata of net panels placed (a) inside net-cages with trash-fish feed, (b) inside net-cages with pellet feed, and (c) outside net-cages (no fish and feed), during the dry season.

higher ($P < 0.05$) cover at the upper and middle strata. *Lyngbya* sp. was present in pellet given net-cages but hardly observed in trash-fish given net-cages.

The wet season favored the continued dominance of *Plumularia* sp. below the upper stratum of panels in net-cages whereas intense spatial competition with *Polysiphonia* sp., *Balanus amphitrite* and *Xenostrobus mangle* occurred at the upper stratum as fouling progressed (Fig. 6a and b). The weekly colonization of *Plumularia* sp., *Polysiphonia* sp., anthozoans, *B. amphitrite*, and *Enteromorpha clathrata* at all depth strata for both feed given net-cages was quite similar. Maximum covers of *X. mangle* on the upper stratum (72.3%) and middle stratum (68.4%) were observed at the 11th and 10th week, respectively.

Plumularia sp. completely dominated the net panels placed outside the net-cages until the completion of the experiment, particularly at the middle and bottom strata (Fig. 6c). *Polysiphonia* sp. and *Xenostrobus mangle* developed slowly at the upper stratum, with maximum covers of 24.4% and 18.7% achieved on the 8th week and 12th week, respectively.

3.5. Biofouling development

In the dry season, the biomass of the sessile net macrofouling organisms inside net-cages had rapidly increased by the 5th–6th week, reaching maximum wet biomass of around 700–850 g and 70–90 g per net panel for sessile and non-sessile organisms, respectively. In the wet season, the net macrofouling rates inside net-cages were slower, reaching comparable biomass between the 9th and 11th week for sessile organisms and after the 10th week for non-sessile organisms. However, mean biomass of sessile and non-sessile organisms on net panels placed outside the cages for the whole period of study did not exceed 300 g and 20 g per net panel, respectively, for both dry and wet seasons. The main treatment effects on the sessile organism biomass of net panels for both dry and wet seasons were as follows: $P > T > O$ ($P < 0.001$), while for non-sessile organism biomass, $P \approx T$ ($P = 0.55$) $> O$ ($P < 0.001$). Although the study suggests that fish cultivation encouraged biofouling of the net panels inside the net-cages, it could not be concluded whether this was due solely to feed input because its effect was confounded by the effect of reduced water flow.

We estimate a mean net biofouling rate of 176 g m^{-2} wk^{-1} outside the net-cage, but fish culture inside the net-cage could increase the net biofouling rate to nearly 400 g m^{-2} wk^{-1} during the dry season. During the wet season, mean net biofouling rates are estimated at 58 g m^{-2} wk^{-1} and 143 g m^{-2} wk^{-1} for outside and inside the net-cage, respectively (Table 3).

4. Discussion

A total of 30 macrofouling species were observed on net panels immersed for 12 weeks inside floating net-cages at the Jaha River estuary. The number is close to that recorded by Cheah and Chua (1979) who found 34 species including non-sessile associates on floating net-cages in the Penang Strait after 2–4 months of submersion. In temperate waters of Shetland, U.K., Braithwaite et al. (2007) recorded only 40 taxa of fouling organisms on salmon cage-nettings immersed over a 10-month period. The dominant 'climax species' as reported in their study, Cheah and Chua (1979), Hodson et al. (2000) and Greene and Grizzle (2007), were overwhelmingly tunicates, ascidians and mussels. Our study however shows the dominance of macroalgae, cnidarians and barnacles during the dry season, but macroalgae, cnidarians and mussels during the wet season. Cosmopolitan macrofouling species such as barnacles and mussels were represented by only a single species each, while other common taxa such as bryozoans were few.

The relatively small number of sessile macrofouling species on cage-nettings is likely due to the pliable and limited attachment surface, as well as shorter immersion time. From a one-year study of biofouling on an offshore oil platform off Bombay waters, Venugopalan and Wagh (1990) reported more than 100 species of mainly sessile organisms. Lin and Shao (2002) reported a higher number of fouling taxa on several types of steel and concrete structures submersed for 12 mo than submersed for 3 mo in Keelung Harbor, Northern Taiwan.

According to Braithwaite and McEvoy (2005), the characteristics of macrofouling community on cage netting material could differ in several respects from that of hard substrates. On the other hand, aquaculture sites appear to attract a large number of non-sessile species occurring in abundance such as amphipods, isopods, copepods and nematodes; similar species however occur infrequently in low numbers at non-aquaculture sites (e.g. Lin and Shao, 2002; Qiu et al., 2003; Yan et al., 2006). Nevertheless, the long-term effect of immersion time on the 'climax fouling community' may not be apparent since the increasingly heavy fouling biomass will likely detach under strong wave action and current, restarting the colonization process again.

The present study shows that species diversity of the netting fouling community did not change seasonally, although the community dominants and their abundance varied between dry and wet seasons (see Fig. 4). It thus highlights the importance of environmental parameters especially salinity and its likely effects on fouling community structure and abundance. Although the effect on organisms' tolerance to low salinity was not studied, low salinity has a depressive effect on the mainly marine species. On the other hand, seasonal factors may also influence the timing of important biological parameters such as reproduction, recruitment, and survival and growth rate of the colonizing organisms (Underwood and Anderson, 1994).

Our study has shown that with stronger water flow of not less than 10 cm s^{-1} as experienced outside the net-cages, the colonization rates of the macroalgae, anthozoans, barnacles, and mussels were very much slower. The growth of these species was otherwise very aggressive in reduced water flow (inside net-cages) suggesting that drag force and shear stress could reduce the retention efficiency of their spores or larvae, or detach the larger individuals. Nevertheless, grazing by wild fish and other predators could also contribute to the slower colonization rates outside the net-cages (Greene and Grizzle, 2007).

Both salinity and current flow appear to modulate the competitive edge of the more aggressive species as is shown in the present study. At lower salinity and in retarded water flow, *Plumularia* sp. consistently dominated inside the net-cages where competition with *Polysiphonia* sp. and *Xenostrobus mangle* occurred only at the upper stratum (0–0.66 m). At higher salinity, the more aggressive *Polysiphonia* sp. and *Balanus amphitrite* soon overwhelmed *Plumularia* sp. Outside the net-cages, *Plumularia* sp. however had almost no competition. Although *Polysiphonia* sp. survived low salinity stress during the wet season, its cover at the upper stratum gradually decreased with the proliferation of *X. mangle* in lower salinity.

Net panel colonization by non-sessile or mobile associates appears to be related to the development of sessile organisms themselves. These mobile organisms should be considered as an important component of macrofouling because of their significant biomass and activity on the cage nettings. Indeed, the succession of species of amphipods on the nettings as well as in tandem with the sessile assemblage has been demonstrated (see Figs. 5 and 6). The relatively higher population of non-sessile associates found inside the net-cages, e.g. amphipods and tanaids, as compared to outside the net-cages, may be their attraction to the microcosm created by

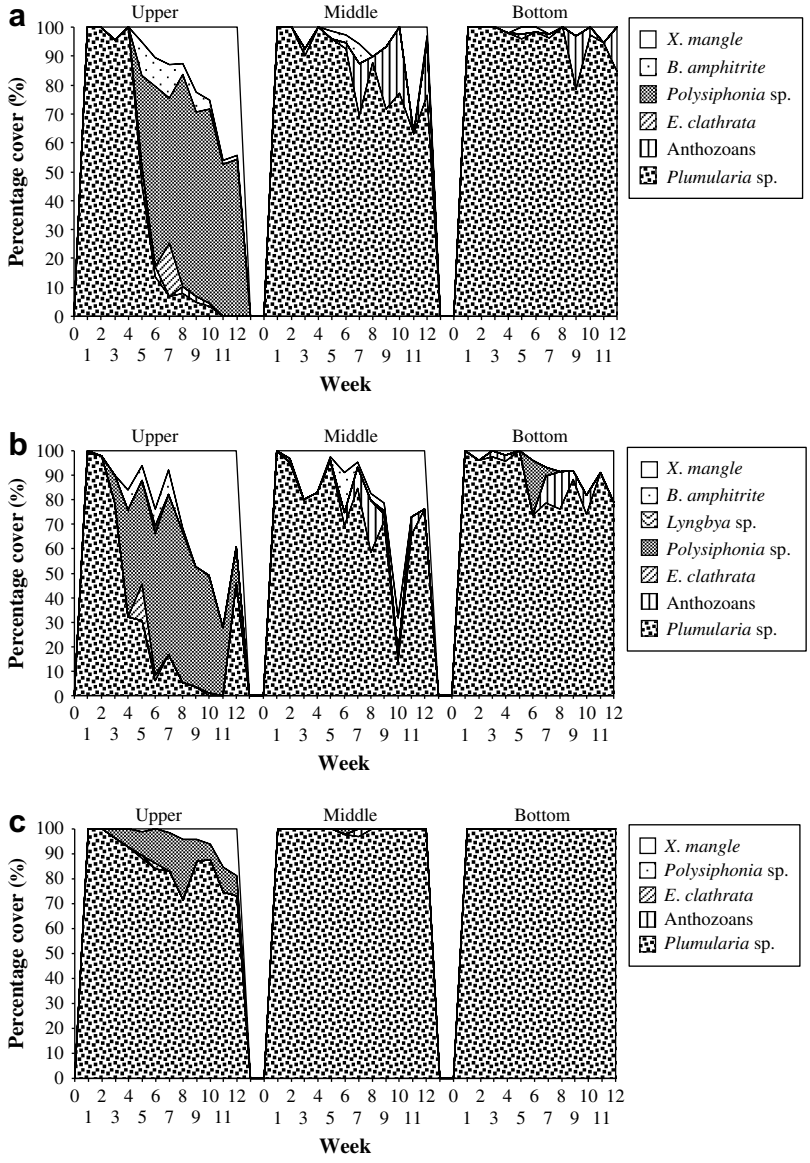

Fig. 6. Temporal changes in percentage cover of sessile macrofouling organisms at the upper, middle and bottom strata of net panels placed (a) inside net-cages with trash-fish feed, (b) inside net-cages with pellet feed, and (c) outside net-cages (no fish and feed), during the wet season.

the sessile assemblage that provides food, habitat space, and shelter.

It has been reported that feed wastage and thus the nutrient loadings are several folds higher for trash-fish feed as compared to pellet feed (Wu, 1995). Therefore, higher feed wastage from trash-fish feed is expected to encourage higher macrofouling rates on net panels. This was however not observed in the present study, instead there were almost similar species of equal abundance and colonization rates for home-made pellet and trash-fish feed (see Fig. 4 and Table 3). Nevertheless, this finding cannot be conclusive

Table 3
Summarized data of highest mean biomass of sessile and non-sessile biofouling organisms by season (wet and dry) and treatment (outside net-cage O, pellet P and trash-fish T). Submersion period in weeks is given in parentheses. Mean biofouling rate based on total weight (g) on 1 m^2 of net panel per week.

	Biofouling rate ($g\,m^{-2}$)			Mean rate ($g\,m^{-2}\,wk^{-1}$)
	Sessile	Non-sessile	Total	
Dry season				
Outside net-cage, no fish and feed, O (4)	670	35	705	176
Fish given pellet feed, P (6)	2095	227.5	2322.5	387
Fish given trash-fish feed, T (5)	1705	175	1880	376
Wet season				
Outside net-cage, no fish and feed, O (11)	610	15	625	58
Fish given pellet feed, P (11)	2465	67.5	2532.5	230
Fish given trash-fish feed, T (12)	1535	192.5	1727.5	143

because our home-made pellet feed had high amount of loose particles and completely disintegrated within 15 min of soakage, and nutrient levels in the experimental cages could be enhanced by nutrient inputs from the surrounding cages.

Nutrient input from metabolite waste of cultivated fish including faecal matter, along with retarded water movement, may also contribute to the higher macrofouling rates inside net-cages. In same farm in Jaha River estuary, Alongi et al. (2003) indicate higher concentrations of NH_4^+, PO_4^3, and $NO_2^- + NO_3^-$ inside the net-cages than in non-cage sites. According to Folke et al. (1994), the higher concentration of nutrient in aquaculture farms is directly available for macroalgae. In the present study, *Polysiphonia* sp. was significantly higher inside the net-cages than outside it, suggesting that nutrient enrichment in retarded water flow could enhance their growth rates. Similarly, nutrient enrichment also encourages phytoplankton blooms which in turn promote the growth of suspension feeders such as *Balanus amphitrite*, anthozoans and *Xenostrobus mangle*.

Fish cultivation with accompanying fish feed inputs and digestive wastes of cultured fish are likely to stimulate a wide range of feeding strategies among non-sessile associates on net panels placed inside the net-cages. Coprophagy commonly observed in copepods, e.g. *Oithona* spp. (Gonzalez and Smetacek, 1994) could well be practiced by macrofouling fauna feeding on the faeces of cultured fish. This is consistent with the present results, where abundance of non-sessile associates particularly *Gammaropsis* sp., *Photis* sp. and *Leptognathia* sp. was significantly higher inside the net-cages than outside. Thus, organic enrichment from fish cultivation affects the development of the non-sessile community. In addition, higher population of macroalgae inside the net-cages could attract particularly amphipods which are also known to feed on them (Duffy, 1990; Cruz-Riveira and Hay, 2000).

Our study concludes that, retarded water flow and inorganic and organic enrichment (via fish feeds and faecal matter) as a result of fish culture enhance the macrofouling assemblage on fish nettings. However, the structure, colonization dynamics, and depth distribution of the macrofouling assemblage are affected by salinity, water depth, and substrate area and immersion period. Due to competition and available larval pools, the macrofouling species exhibit vertical distribution and succession which are modulated by the effects of fish culture. From the study, we recommend the rearing of young fish in fine-meshed cage-nets during the wet months in order to reduce the frequency of net cleaning for several months. Future study on the separate effect of water flow velocity, organic and nutrient enrichment on net biofouling rate would be beneficial in terms of siting and spacing of fish-cage units and choice of fish feed.

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APPENDIX 3

PCA Analysis on Macrofouling Dynamics in Relation to Fish Rearing and Season at Jaha

Appendix 3

PCA Analysis on Macrofouling Dynamics in Relation to Fish Rearing and Season at Jaha

WCanoImp produced data file

PCA	Canonical axes:	0	Covariables:	0	Scaling:	2
Cent./stand.	by samples:	0	0	by species:	1	1

No transformation

Spec: Species scores (adjusted for species variance)

N	NAME	AX1	AX2	AX3	AX4	WEIGHT	1
	EIG	0.3244	0.117	0.0746	0.0628		
1	Gam	0.9098	0.0737	-0.0003	0.2109	1	1
2	Pho	0.9718	0.0691	0.0722	-0.0263	1	1
3	Cor	0.82	0.2416	0.1779	-0.2139	1	1
4	Git	0.8415	0.2185	0.1798	-0.1681	1	1
5	Chi	0.5764	-0.5883	-0.0569	-0.2608	1	1
6	Lep	0.9208	0.0388	0.0184	0.1862	1	1
7	Nem	0.787	0.4189	0.0505	0.2591	1	1
8	Eut	0.564	0.694	0.1688	0.2224	1	1
9	Tig	0.106	0.3553	0.1614	-0.0464	1	1
10	Oit	-0.159	0.0856	-0.2407	0.2323	1	1
11	Aca	-0.2407	-0.3276	0.5381	-0.1256	1	1
12	Mic	-0.2799	-0.1542	0.7217	-0.0405	1	1
13	Onc	-0.1466	-0.0694	-0.0834	0.3218	1	1
14	Par	-0.2769	-0.2094	0.1421	0.6635	1	1
15	Mic 2	-0.3426	-0.1946	0.7672	-0.0677	1	1
16	Sap	-0.2354	0.0992	-0.0046	-0.1153	1	1
17	Par 2	-0.3986	-0.2362	0.6612	-0.003	1	1
18	Dor	0.0737	0.5352	0.1623	-0.1511	1	1
19	Dor2	0.6877	0.2146	0.1859	0.2105	1	1
20	Per	0.8522	0.1846	0.1874	0.1644	1	1
21	Ter	-0.1136	-0.1705	-0.0713	-0.3399	1	1
22	Bra	0.2074	-0.2605	0.1516	0.6424	1	1
23	Cir	0.4477	0.2512	0.2256	-0.3138	1	1
24	Plu	-0.8198	0.3499	-0.0246	0.1619	1	1
25	Ant	0.7078	-0.5989	-0.0729	-0.1615	1	1
26	Ent	0.6502	-0.4849	-0.0122	0.3371	1	1
27	Pol	0.7508	-0.4395	-0.0256	-0.06	1	1
28	Lyn	-0.2033	-0.1316	0.3	0.2741	1	1
29	Bal	0.7552	-0.4584	-0.003	-0.1292	1	1
30	Xen	0.2787	0.59	0.164	-0.214	1	1

WCanoImp produced data file

PCA	Canonical axes:	0	Covariables:	0	Scaling:	2
Cent./stand.	by samples:	0	0	by species:	1	1

No transformation

Samp: Sample scores

N	NAME	AX1	AX2	AX3	AX4	WEIGHT	1
	EIG	0.3244	0.117	0.0746	0.0628		
1	D1C	-0.4801	0.653	-0.7509	-0.1987	1	1
2	D1P	0.3075	1.676	-0.0064	0.8691	1	1
3	D1T	-0.2278	1.0327	-0.2515	0.1948	1	1
4	D2C	-0.6275	-0.0224	-0.1706	0.012	1	1
5	D2P	-0.6744	-0.1614	0.769	0.3048	1	1

6	D2T	-0.4528	0.1686	-0.749	0.2688	1	1
7	D3C	-1.2664	-1.1879	4.421	0.6851	1	1
8	D3P	0.6577	0.0638	-0.6749	0.7028	1	1
9	D3T	1.6356	-0.6178	0.7291	5.3775	1	1
10	D4C	-0.6945	-0.2895	-0.2172	-0.0909	1	1
11	D4P	0.8727	-1.3603	-0.7077	0.7872	1	1
12	D4T	1.0308	-1.084	-0.8143	0.3827	1	1
13	D5C	-0.5853	-0.314	-0.2109	0.5178	1	1
14	D5P	1.4748	-1.2579	-0.3432	-0.6927	1	1
15	D5T	2.5259	-0.1362	0.3945	0.3943	1	1
16	D6C	-0.7493	-0.3907	0.2797	-0.416	1	1
17	D6P	2.0279	-2.3653	0.6616	-1.3103	1	1
18	D6T	1.4408	-2.2232	-0.5099	0.1185	1	1
19	D7C	-0.8237	-0.3284	-0.4039	1.6817	1	1
20	D7P	1.6215	-2.088	-0.6553	-1.8532	1	1
21	D7T	1.4175	-0.8256	-0.2055	-0.6903	1	1
22	D8C	-0.7762	-0.6925	1.2794	1.6326	1	1
23	D8P	2.1343	-1.01	0.162	-0.8043	1	1
24	D8T	0.5637	-1.1783	-0.6037	-1.0802	1	1
25	W10C	-0.9546	-0.1918	-0.9248	-0.1656	1	1
26	W10P	0.6461	1.2303	0.3121	-0.9076	1	1
27	W10T	0.5755	1.2735	-0.0967	-0.1495	1	1
28	W11C	-0.8649	-0.1987	-0.2693	-0.3592	1	1
29	W11P	0.2902	1.3681	0.159	-0.9122	1	1
30	W11T	1.5064	2.0194	0.647	-0.0958	1	1
31	W12C	-1.017	-0.2587	0.8941	-1.358	1	1
32	W12P	-0.0889	1.0482	-0.2658	-1.1828	1	1
33	W12T	1.5168	2.9295	1.3966	-1.1161	1	1
34	W1C	-0.8321	0.1571	-0.9396	-0.2117	1	1
35	W1P	-0.9493	-0.0839	-1.0281	-0.1337	1	1
36	W1T	-0.9955	-0.1312	-1.0609	-0.1648	1	1
37	W2C	-1.5136	-1.1486	3.4575	-0.9918	1	1
38	W2P	-0.3465	0.4486	-0.6863	0.304	1	1
39	W2T	-0.8167	-0.0972	-0.9499	-0.3568	1	1
40	W3C	-1.1793	-0.5003	0.8877	-0.145	1	1
41	W3P	-0.2367	0.4167	-0.1594	0.1824	1	1
42	W3T	-0.5104	0.4984	-0.6773	0.0802	1	1
43	W4C	-1.0724	-0.1647	-1.2108	-0.3941	1	1
44	W4P	0.1667	0.9611	-0.8809	2.0765	1	1
45	W4T	-0.74	0.1603	-0.6893	-0.1666	1	1
46	W5C	-1.0355	-0.3047	-0.3421	0.1483	1	1
47	W5P	-0.5249	0.1845	-0.9129	0.4164	1	1
48	W5T	-0.6029	0.047	-0.2067	-0.1256	1	1
49	W6C	-1.0356	-0.4691	-0.1002	0.1741	1	1
50	W6P	0.5271	0.3877	-0.2641	0.5293	1	1
51	W6T	-0.3262	0.1817	-0.6714	-0.6971	1	1
52	W7C	-0.9298	-0.2436	-0.422	0.0601	1	1
53	W7P	0.5583	1.3193	1.596	-0.28	1	1
54	W7T	0.071	0.3101	-0.4418	-0.3265	1	1
55	W8C	-0.9695	-0.5567	0.5003	-0.2313	1	1
56	W8P	0.7764	0.7467	1.6728	-0.1709	1	1
57	W8T	0.19	0.5411	-0.4294	0.4094	1	1
58	W9C	-0.9561	-0.5763	0.0132	-0.2243	1	1
59	W9P	0.3479	0.693	0.1039	0.3922	1	1
60	W9T	0.9737	1.9423	0.5679	-0.6992	1	1
61	ORIGIN	-0.9731	-0.685	-1.0702	-1.0426	0	0

WCanoImp produced data file

PCA	Canonical axes:	0	Covariables:	0	Scaling:	2
Cent./stand.	by samples:	0	0	by species:	1	1

No transformation

CFit: Cumulative fit per species as fraction of variance of species

N	NAME	AX1	AX2	AX3	AX4	VAR(y)	% EXPL
	FR FITTED	0.3244	0.117	0.0746	0.0628		
1	Gam	0.8278	0.8332	0.8332	0.8777	1	0
2	Pho	0.9444	0.9492	0.9544	0.9551	1	0
3	Cor	0.6724	0.7307	0.7624	0.8081	1	0
4	Git	0.7081	0.7558	0.7881	0.8164	1	0
5	Chi	0.3322	0.6783	0.6815	0.7495	1	0
6	Lep	0.8479	0.8494	0.8498	0.8845	1	0
7	Nem	0.6194	0.7948	0.7974	0.8645	1	0
8	Eut	0.318	0.7997	0.8282	0.8777	1	0
9	Tig	0.0112	0.1374	0.1635	0.1656	1	0
10	Oit	0.0253	0.0326	0.0906	0.1445	1	0
11	Aca	0.058	0.1653	0.4548	0.4706	1	0
12	Mic	0.0783	0.1021	0.623	0.6247	1	0
13	Onc	0.0215	0.0263	0.0333	0.1368	1	0
14	Par	0.0767	0.1205	0.1407	0.581	1	0
15	Mic 2	0.1174	0.1553	0.7438	0.7484	1	0
16	Sap	0.0554	0.0652	0.0653	0.0785	1	0
17	Par 2	0.1589	0.2146	0.6518	0.6518	1	0
18	Dor	0.0054	0.2918	0.3182	0.341	1	0
19	Dor2	0.4729	0.519	0.5535	0.5978	1	0
20	Per	0.7263	0.7604	0.7955	0.8225	1	0
21	Ter	0.0129	0.042	0.0471	0.1626	1	0
22	Bra	0.043	0.1109	0.1339	0.5466	1	0
23	Cir	0.2004	0.2636	0.3145	0.4129	1	0
24	Plu	0.672	0.7945	0.7951	0.8213	1	0
25	Ant	0.501	0.8597	0.865	0.8911	1	0
26	Ent	0.4227	0.6579	0.658	0.7717	1	0
27	Pol	0.5636	0.7568	0.7574	0.761	1	0
28	Lyn	0.0413	0.0586	0.1487	0.2238	1	0
29	Bal	0.5703	0.7804	0.7804	0.7971	1	0
30	Xen	0.0777	0.4258	0.4527	0.4984	1	0

WCanoImp produced data file

PCA	Canonical axes:	0	Covariables:	0	Scaling:	2
Cent./stand.	by samples:	0	0	by species:	1	1

No transformation

SqRL: Squared residual length per sample with s axes (s=1...4)

N	NAME	AX1	AX2	AX3	AX4	SQLENG	% FIT
	FR FITTED	0.3244	0.117	0.0746	0.0628		
1	D1C	0.3427	0.2928	0.2507	0.2483	0.42	40.53
2	D1P	0.9847	0.6559	0.6559	0.6085	1.02	40.07
3	D1T	0.4116	0.2868	0.2821	0.2797	0.43	34.72
4	D2C	0.1209	0.1208	0.1187	0.1187	0.25	52.27
5	D2P	0.7102	0.7072	0.6631	0.6572	0.86	23.38
6	D2T	0.5862	0.5828	0.541	0.5364	0.65	17.81
7	D3C	1.8741	1.7089	0.2508	0.2213	2.39	90.76
8	D3P	0.8994	0.8989	0.8649	0.8339	1.04	19.8
9	D3T	2.5218	2.4771	2.4374	0.6206	3.39	81.69
10	D4C	0.217	0.2072	0.2036	0.2031	0.37	45.61
11	D4P	0.7192	0.5026	0.4652	0.4263	0.97	55.88
12	D4T	0.782	0.6445	0.595	0.5858	1.13	48

13	D5C	0.2641	0.2525	0.2492	0.2324	0.38	38.07
14	D5P	0.4813	0.2961	0.2873	0.2571	1.19	78.33
15	D5T	0.4397	0.4375	0.4259	0.4161	2.51	83.42
16	D6C	0.2189	0.2011	0.1952	0.1844	0.4	54.03
17	D6P	1.4297	0.7749	0.7423	0.6344	2.76	77.05
18	D6T	1.0188	0.4403	0.4209	0.42	1.69	75.18
19	D7C	1.0267	1.0141	1.0019	0.8242	1.25	33.89
20	D7P	1.5906	1.0803	1.0483	0.8325	2.44	65.93
21	D7T	0.3361	0.2563	0.2532	0.2232	0.99	77.4
22	D8C	1.2599	1.2038	1.0817	0.9142	1.46	37.18
23	D8P	0.6602	0.5408	0.5388	0.4982	2.14	76.7
24	D8T	0.514	0.3515	0.3244	0.251	0.62	59.32
25	W10C	0.193	0.1887	0.1249	0.1232	0.49	74.79
26	W10P	0.716	0.5389	0.5316	0.4799	0.85	43.64
27	W10T	0.5603	0.3705	0.3698	0.3684	0.67	44.83
28	W11C	0.2298	0.2252	0.2198	0.2117	0.47	55.2
29	W11P	0.715	0.496	0.4941	0.4418	0.74	40.49
30	W11T	1.0841	0.6068	0.5756	0.575	1.82	68.41
31	W12C	0.7358	0.7279	0.6683	0.5524	1.07	48.43
32	W12P	0.7329	0.6043	0.599	0.5111	0.74	30.5
33	W12T	2.0372	1.0329	0.8873	0.8091	2.78	70.93
34	W1C	0.208	0.2052	0.1393	0.1365	0.43	68.46
35	W1P	0.2144	0.2136	0.1347	0.1336	0.51	73.63
36	W1T	0.2238	0.2218	0.1378	0.1361	0.55	75.04
37	W2C	1.4468	1.2924	0.4006	0.3388	2.19	84.53
38	W2P	0.2115	0.188	0.1528	0.147	0.25	41.31
39	W2T	0.1585	0.1574	0.0901	0.0821	0.37	78.11
40	W3C	0.2851	0.2558	0.197	0.1957	0.74	73.42
41	W3P	0.2196	0.1993	0.1974	0.1953	0.24	17.87
42	W3T	0.9051	0.876	0.8418	0.8414	0.99	14.98
43	W4C	0.2349	0.2317	0.1224	0.1126	0.61	81.48
44	W4P	1.4111	1.303	1.2451	0.9742	1.42	31.4
45	W4T	0.4939	0.4909	0.4555	0.4537	0.67	32.44
46	W5C	0.1456	0.1348	0.126	0.1247	0.49	74.74
47	W5P	0.2867	0.2827	0.2206	0.2097	0.38	44.25
48	W5T	0.1496	0.1493	0.1461	0.1451	0.27	45.74
49	W6C	0.1981	0.1723	0.1716	0.1697	0.55	68.92
50	W6P	0.4503	0.4327	0.4275	0.4099	0.54	24.15
51	W6T	0.2581	0.2543	0.2206	0.1901	0.29	35.04
52	W7C	0.1835	0.1766	0.1633	0.1631	0.46	64.85
53	W7P	1.3986	1.1949	1.0048	0.9999	1.5	33.33
54	W7T	0.1716	0.1604	0.1458	0.1391	0.17	19.71
55	W8C	0.4568	0.4205	0.4019	0.3985	0.76	47.68
56	W8P	1.0625	0.9972	0.7885	0.7866	1.26	37.47
57	W8T	0.8462	0.8119	0.7982	0.7876	0.86	8.19
58	W9C	0.3389	0.3	0.3	0.2968	0.64	53.28
59	W9P	0.6712	0.615	0.6142	0.6046	0.71	14.91
60	W9T	1.4925	1.051	1.0269	0.9962	1.8	44.66

APPENDIX 4

Summary of 4-Way ANOVA and *Post Hoc* Test Results on the Effects of Season (Dry, Wet), Depth (Upper, Middle, Bottom), Fish Feed (P = Pellet, T = Trash-Fish, O = Outside Cages) and Immersion Time (Wk 1, 2, 3…,8) on Percentage Cover of Each Sessile Biofouling Species at Jaha

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Summary of 4-Way ANOVA and *Post Hoc* Test Results on the Effects of Season (Dry, Wet), Depth (Upper, Middle, Bottom), Fish Feed (P = Pellet, T = Trash-Fish, O = Outside Cages) and Immersion Time (Wk 1, 2, 3…,8) on Percentage Cover of Each Sessile Biofouling Species at Jaha

a. *Plumularia* sp.

ANOVA results

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Season	1	218793.3281	1143	246.4978	887.6075	0.0000
Depth	2	32982.9727	1143	246.4978	133.8064	0.0000
Feed	2	189758.3906	1143	246.4978	769.8177	0.0000
Week	7	9516.4453	1143	246.4978	38.6066	0.0000
Season x Depth	2	1428.5864	1143	246.4978	5.7955	0.0031
Season x Feed	2	6809.1841	1143	246.4978	27.6237	0.0000
Depth x Feed	4	2263.2559	1143	246.4978	9.1816	0.0000
Season x Week	7	2127.9661	1143	246.4978	8.6328	0.0000
Depth x Week	14	3808.8064	1143	246.4978	15.4517	0.0000
Feed x Week	14	7424.7881	1143	246.4978	30.1211	0.0000
Sesaon x Depth x Feed	4	2657.5544	1143	246.4978	10.7813	0.0000
Season x Depth x Week	14	1425.3149	1143	246.4978	5.7823	0.0000
Season x Feed x Week	14	2662.7991	1143	246.4978	10.8025	0.0000
Depth x Feed x Week	28	626.8259	1143	246.4978	2.5429	0.0000
Season x Depth x Feed x Week	28	731.7539	1143	246.4978	2.9686	0.0000

Post hoc test results (Student – Newman – Keuls test)

				{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}	{17}	{18}
Season	Depth	Feed		16.51	16.01	49.33	36.68	42.63	77.01	36.68	54.97	77.01	45.80	58.75	89.44	83.32	91.94	99.31	89.74	98.57	100.00
Dry	Upper	Pellet	{1}		0.5902	0.0000	0.0574	0.0749	0.0000	0.0775	0.0924	0.0000	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Dry	Upper	Trash fish	{2}	0.5902		0.0000	0.0391	0.0227	0.0000	0.0348	0.0341	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Dry	Upper	Outside cages	{3}	0.0000	0.0000		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0039	0.6775	0.0000	0.0316	0.0000	0.0000	0.1364	0.0000	0.0000
Dry	Middle	Pellet	{4}	0.0574	0.0391	0.0000		0.9065	0.0000	0.7924	0.9054	0.0000	0.1068	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Dry	Middle	Trash fish	{5}	0.0749	0.0227	0.0000	0.9065		0.0000	0.9106	0.8020	0.0000	0.0790	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Dry	Middle	Outside cages	{6}	0.0000	0.0000	0.0000	0.0000	0.0000		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0803	0.0000	0.0000	0.1178	0.0000
Dry	Bottom	Pellet	{7}	0.0775	0.0348	0.0000	0.7924	0.9106	0.0000		0.8718	0.0000	0.1320	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Dry	Bottom	Trash fish	{8}	0.0924	0.0341	0.0000	0.9054	0.8020	0.0000	0.8718		0.0000	0.1104	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Dry	Bottom	Outside cages	{9}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.0000	0.0000	0.1419	0.0000	0.0002	0.0000	0.0000	0.0002	0.0000
Wet	Upper	Pellet	{10}	0.0002	0.0000	0.0039	0.1068	0.0790	0.0000	0.1320	0.1104	0.0000		0.0028	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Wet	Upper	Trash fish	{11}	0.0000	0.0000	0.6775	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0028		0.0000	0.0530	0.0000	0.0000	0.1357	0.0000	0.0000
Wet	Upper	Outside cages	{12}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1419	0.0000	0.0000		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Wet	Middle	Pellet	{13}	0.0000	0.0000	0.0316	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0530	0.0000		0.0000	0.0000	0.4073	0.0000	0.0000
Wet	Middle	Trash fish	{14}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0803	0.0000	0.0000	0.0002	0.0000	0.0000	0.0000	0.0000		0.0000	0.0000	0.8191	0.0000
Wet	Middle	Outside cages	{15}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.0000	0.0000	0.9732
Wet	Bottom	Pellet	{16}	0.0000	0.0000	0.1364	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1357	0.0000	0.4073	0.0000	0.0000		0.0000	0.0000
Wet	Bottom	Trash fish	{17}	0.0000	0.0000	0.0000	0.0000	0.0000	0.1178	0.0000	0.0000	0.0002	0.0000	0.0000	0.0000	0.0000	0.8191	0.0000	0.0000		0.0000
Wet	Bottom	Outside cages	{18}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9732	0.0000	0.0000	

b. Anthozoans (unidentified sea anemone)

ANOVA results

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Season	1	17917.4238	1143	29.3708	610.0427	0.0000
Depth	2	1681.2854	1143	29.3708	57.2435	0.0000
Feed	2	1505.0636	1143	29.3708	51.2436	0.0000
Week	7	926.3090	1143	29.3708	31.5385	0.0000
Season x Depth	2	1113.7343	1143	29.3708	37.9198	0.0000
Season x Feed	2	831.5385	1143	29.3708	28.3118	0.0000
Depth x Feed	4	409.7498	1143	29.3708	13.9509	0.0000
Season x Week	7	675.2835	1143	29.3708	22.9917	0.0000
Depth x Week	14	186.9307	1143	29.3708	6.3645	0.0000
Feed x Week	14	233.4819	1143	29.3708	7.9495	0.0000

Season x Depth x Feed	4	244.7385	1143	29.3708	8.3327	0.0000
Season x Depth x Week	14	87.4462	1143	29.3708	2.9773	0.0002
Season x Feed x Week	14	200.9571	1143	29.3708	6.8421	0.0000
Depth x Feed x Week	28	51.1536	1143	29.3708	1.7417	0.0100
Season x Depth x Feed x Week	28	63.6154	1143	29.3708	2.1659	0.0004

Post hoc test results (Student – Newman – Keuls test)

				{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}	{17}	{18}
Season	Depth	Feed		16.5068	8.3356	6.5647	36.6751	32.6070	8.3218	36.6751	32.7080	8.3218	0.2691	1.4494	0.1425	4.3488	3.0318	0.3865	3.9110	0.3841	0.0000
Dry	Upper	Pellet	{1}		0.3704	0.5961	0.0000	0.0000	0.3160	0.0000	0.0000	0.7255	0.0058	0.0244	0.0065	0.0966	0.0543	0.0073	0.0551	0.0066	0.0047
Dry	Upper	Trash fish	{2}	0.3704		0.5238	0.0000	0.0000	0.7734	0.0000	0.0000	0.3948	0.0000	0.0001	0.0000	0.0093	0.0012	0.0000	0.0007	0.0000	0.0000
Dry	Upper	Outside cages	{3}	0.5961	0.5238		0.0000	0.0000	0.5168	0.0000	0.0000	0.8155	0.0009	0.0061	0.0010	0.0726	0.0233	0.0013	0.0187	0.0011	0.0006
Dry	Middle	Pellet	{4}	0.0000	0.0000	0.0000		0.6777	0.0000	0.6595	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Dry	Middle	Trash fish	{5}	0.0000	0.0000	0.0000	0.6777		0.0000	0.6892	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Dry	Middle	Outside cages	{6}	0.3160	0.7734	0.5168	0.0000	0.0000		0.0000	0.0000	0.4900	0.0000	0.0000	0.0000	0.0049	0.0005	0.0000	0.0003	0.0000	0.0000
Dry	Bottom	Pellet	{7}	0.0000	0.0000	0.0000	0.6595	0.6892	0.0000		0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Dry	Bottom	Trash fish	{8}	0.0000	0.0000	0.0000	0.0000	0.0002	0.0000	0.0001		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Dry	Bottom	Outside cages	{9}	0.7255	0.3948	0.8155	0.0000	0.0000	0.4900	0.0000	0.0000		0.0004	0.0035	0.0004	0.0724	0.0180	0.0007	0.0126	0.0005	0.0003
Wet	Upper	Pellet	{10}	0.0058	0.0000	0.0009	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004		0.8965	0.9747	0.4041	0.7469	0.9725	0.7822	0.9142	0.9840
Wet	Upper	Trash fish	{11}	0.0244	0.0001	0.0061	0.0000	0.0000	0.0000	0.0000	0.0000	0.0035	0.8965		0.9488	0.5534	0.7836	0.6336	0.6570	0.8234	0.9531
Wet	Upper	Outside cages	{12}	0.0065	0.0000	0.0010	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.9747	0.9488		0.4492	0.8029	0.9941	0.8478	0.9894	0.8892
Wet	Middle	Pellet	{13}	0.0966	0.0093	0.0726	0.0000	0.0000	0.0049	0.0000	0.0000	0.0724	0.4041	0.5534	0.4492		0.5161	0.3785	0.6588	0.3967	0.4138
Wet	Middle	Trash fish	{14}	0.0543	0.0012	0.0233	0.0000	0.0000	0.0005	0.0000	0.0000	0.0180	0.7469	0.7836	0.8029	0.5161		0.6634	0.8249	0.7163	0.7868
Wet	Middle	Outside cages	{15}	0.0073	0.0000	0.0013	0.0000	0.0000	0.0000	0.0000	0.0000	0.0007	0.9725	0.6336	0.9941	0.3785	0.6634		0.6271	0.9067	0.9948
Wet	Bottom	Pellet	{16}	0.0551	0.0007	0.0187	0.0000	0.0000	0.0003	0.0000	0.0000	0.0126	0.7822	0.6570	0.8478	0.6588	0.8249	0.6271		0.7271	0.8444
Wet	Bottom	Trash fish	{17}	0.0066	0.0000	0.0011	0.0000	0.0000	0.0000	0.0000	0.0000	0.0005	0.9142	0.8234	0.9894	0.3967	0.7163	0.9067	0.7271		0.9924
Wet	Bottom	Outside cages	{18}	0.0047	0.0000	0.0006	0.0000	0.0000	0.0000	0.0000	0.0000	0.0003	0.9840	0.9531	0.8892	0.4138	0.7868	0.9948	0.8444	0.9924	

c. *Balanus amphitrite*

ANOVA results

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level

Season	1	1981.7456	1143	18.6114	106.4804	0.0000
Depth	2	47.5467	1143	18.6114	2.5547	0.0782
Feed	2	607.3171	1143	18.6114	32.6315	0.0000
Week	7	412.9334	1143	18.6114	22.1872	0.0000
Season x Depth	2	157.9882	1143	18.6114	8.4888	0.0002
Season x Feed	2	71.0320	1143	18.6114	3.8166	0.0223
Depth x Feed	4	10.6991	1143	18.6114	0.5749	0.6809
Season x Week	7	104.0211	1143	18.6114	5.5891	0.0000
Depth x Week	14	14.0790	1143	18.6114	0.7565	0.7174
Feed x Week	14	89.2165	1143	18.6114	4.7937	0.0000
Season x Depth x Feed	4	20.4863	1143	18.6114	1.1007	0.3548
Season x Depth x Week	14	25.3059	1143	18.6114	1.3597	0.1658
Season x Feed x Week	14	66.3954	1143	18.6114	3.5675	0.0000
Depth x Feed x Week	28	26.8471	1143	18.6114	1.4425	0.0642
Season x Depth x Feed x Week	28	21.6666	1143	18.6114	1.1642	0.2545

Post hoc test results (Student – Newman – Keuls test)

				{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}	{17}	{18}
Season	Depth	Feed		5.86	5.01	2.34	13.45	11.01	4.07	13.45	10.63	4.07	4.13	4.67	0.00	2.88	1.52	0.00	3.27	0.52	0.00
Dry	Upper	Pellet	{1}		0.3899	0.0122	0.0337	0.3361	0.2632	0.2883	0.4222	0.0091	0.1337	0.3396	0.0000	0.0096	0.0010	0.0000	0.0084	0.0001	0.0000
Dry	Upper	Trash fish	{2}	0.3899		0.2260	0.0008	0.1048	0.6087	0.0392	0.6123	0.2404	0.6530	0.8466	0.0036	0.2243	0.0751	0.0031	0.2483	0.0132	0.0026
Dry	Upper	Outside cages	{3}	0.0122	0.2260		0.0000	0.0004	0.3934	0.0000	0.0989	0.9735	0.3536	0.2739	0.5732	0.8864	0.9065	0.5108	0.9907	0.6746	0.4419
Dry	Middle	Pellet	{4}	0.0337	0.0008	0.0000		0.1862	0.0001	0.2271	0.0040	0.0000	0.0000	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Dry	Middle	Trash fish	{5}	0.3361	0.1048	0.0004	0.1862		0.0429	0.5868	0.1816	0.0002	0.0108	0.0542	0.0000	0.0003	0.0000	0.0000	0.0002	0.0000	0.0000
Dry	Middle	Outside cages	{6}	0.2632	0.6087	0.3934	0.0001	0.0429		0.0114	0.5650	0.4689	0.7942	0.9697	0.0211	0.4233	0.2181	0.0180	0.4980	0.0568	0.0151
Dry	Bottom	Pellet	{7}	0.2883	0.0392	0.0000	0.2271	0.5868	0.0114		0.0962	0.0000	0.0018	0.0135	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Dry	Bottom	Trash fish	{8}	0.4222	0.6123	0.0989	0.0040	0.1816	0.5650	0.0962		0.0915	0.4555	0.7159	0.0005	0.0895	0.0188	0.0004	0.0905	0.0023	0.0003
Dry	Bottom	Outside cages	{9}	0.0091	0.2404	0.9735	0.0000	0.0002	0.4689	0.0000	0.0915		0.6595	0.3989	0.5910	0.9379	0.7905	0.5071	0.9374	0.5864	0.4111
Wet	Upper	Pellet	{10}	0.1337	0.6530	0.3536	0.0000	0.0108	0.7942	0.0018	0.4555	0.6595		0.5427	0.1096	0.5325	0.4646	0.0922	0.7357	0.1935	0.0755
Wet	Upper	Trash fish	{11}	0.3396	0.8466	0.2739	0.0002	0.0542	0.9697	0.0135	0.7159	0.3989	0.5427		0.0205	0.3347	0.1940	0.0172	0.4426	0.0516	0.0141
Wet	Upper	Outside cages	{12}	0.0000	0.0036	0.5732	0.0000	0.0000	0.0211	0.0000	0.0005	0.5910	0.1096	0.0205		0.6099	0.8151	1.0000	0.5595	0.9649	1.0000
Wet	Middle	Pellet	{13}	0.0096	0.2243	0.8864	0.0000	0.0003	0.4233	0.0000	0.0895	0.9379	0.5325	0.3347	0.6099		0.8846	0.5379	0.9866	0.6654	0.4560
Wet	Middle	Trash fish	{14}	0.0010	0.0751	0.9065	0.0000	0.0000	0.2181	0.0000	0.0188	0.7905	0.4646	0.1940	0.8151	0.8846		0.6996	0.5654	0.5419	0.5242

Wet	Middle	Outside cages	{15}	0.0000	0.0031	0.5108	0.0000	0.0000	0.0180	0.0000	0.0004	0.5071	0.0922	0.0172	1.0000	0.5379	0.6996		0.4600	0.8837	1.0000
Wet	Bottom	Pellet	{16}	0.0084	0.2483	0.9907	0.0000	0.0002	0.4980	0.0000	0.0905	0.9374	0.7357	0.4426	0.5595	0.9866	0.5654	0.4600		0.4623	0.3459
Wet	Bottom	Trash fish	{17}	0.0001	0.0132	0.6746	0.0000	0.0000	0.0568	0.0000	0.0023	0.5864	0.1935	0.0516	0.9649	0.6654	0.5419	0.8837	0.4623		0.6357
Wet	Bottom	Outside cages	{18}	0.0000	0.0026	0.4419	0.0000	0.0000	0.0151	0.0000	0.0003	0.4111	0.0755	0.0141	1.0000	0.4560	0.5242	1.0000	0.3459	0.6357	

d. *Polysiphonia* sp.

ANOVA results

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Season	1	6697.5981	1142	75.6340	88.5527	0.0000
Depth	2	49722.0625	1142	75.6340	657.4033	0.0000
Feed	2	7856.2080	1142	75.6340	103.8713	0.0000
Week	7	3154.3906	1142	75.6340	41.7060	0.0000
Season x Depth	2	2922.9329	1142	75.6340	38.6457	0.0000
Season x Feed	2	2059.8684	1142	75.6340	27.2347	0.0000
Depth x Feed	4	6382.6255	1142	75.6340	84.3883	0.0000
Season x Week	7	735.0406	1142	75.6340	9.7184	0.0000
Depth x Week	14	3110.9773	1142	75.6340	41.1320	0.0000
Feed x Week	14	724.7917	1142	75.6340	9.5829	0.0000
Season x Depth x Feed	4	1205.9277	1142	75.6340	15.9442	0.0000
Season x Depth x Week	14	519.5339	1142	75.6340	6.8690	0.0000
Season x Feed x Week	14	393.9845	1142	75.6340	5.2091	0.0000
Depth x Feed x Week	28	737.5299	1142	75.6340	9.7513	0.0000
Season x Depth x Feed x Week	28	296.9136	1142	75.6340	3.9257	0.0000

Post hoc test results (Student – Newman – Keuls test)

				{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}	{17}	{18}
Season	Depth	Feed		53.30	57.26	7.71	8.92	12.18	2.04	8.92	1.07	2.04	35.32	27.14	9.24	0.24	0.00	0.31	0.00	0.00	0.00
Dry	Upper	Pellet	{1}		0.5808	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Dry	Upper	Trash fish	{2}	0.5808		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Dry	Upper	Outside cages	{3}	0.0000	0.0000		0.5812	0.7948	0.0523	0.0605	0.0154	0.0521	0.0000	0.0000	0.2661	0.0203	0.0441	0.0219	0.0365	0.0294	0.0229
Dry	Middle	Pellet	{4}	0.0000	0.0000	0.5812		0.6957	0.0760	0.2215	0.0404	0.1930	0.0000	0.0000	0.1514	0.0706	0.1648	0.0663	0.1372	0.1104	0.0849

Dry	Middle	Trash fish	{5}	0.0000	0.0000	0.7948	0.6957		0.0478	0.0306	0.0106	0.0265	0.0000	0.0000	0.1959	0.0116	0.0226	0.0137	0.0190	0.0156	0.0124
Dry	Middle	Outside cages	{6}	0.0000	0.0000	0.0523	0.0760	0.0478		0.9971	0.5141	0.9941	0.0000	0.0000	0.0010	0.8430	0.9880	0.7719	0.9757	0.9516	0.9047
Dry	Bottom	Pellet	{7}	0.0000	0.0000	0.0605	0.2215	0.0306	0.9971		1.0000	1.0000	0.0000	0.0000	0.0002	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Dry	Bottom	Trash fish	{8}	0.0000	0.0000	0.0154	0.0404	0.0106	0.5141	1.0000		1.0000	0.0000	0.0000	0.0001	0.9839	1.0000	0.9736	0.9999	0.9994	0.9959
Dry	Bottom	Outside cages	{9}	0.0000	0.0000	0.0521	0.1930	0.0265	0.9941	1.0000	1.0000		0.0000	0.0000	0.0002	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Wet	Upper	Pellet	{10}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.0122	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Wet	Upper	Trash fish	{11}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0122		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Wet	Upper	Outside cages	{12}	0.0000	0.0000	0.2661	0.1514	0.1959	0.0010	0.0002	0.0001	0.0002	0.0000	0.0000		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Wet	Middle	Pellet	{13}	0.0000	0.0000	0.0203	0.0706	0.0116	0.8430	1.0000	0.9839	1.0000	0.0000	0.0000	0.0001		1.0000	0.8897	0.9999	0.9983	0.9563
Wet	Middle	Trash fish	{14}	0.0000	0.0000	0.0441	0.1648	0.0226	0.9880	1.0000	1.0000	1.0000	0.0000	0.0000	0.0001	1.0000		1.0000	1.0000	1.0000	1.0000
Wet	Middle	Outside cages	{15}	0.0000	0.0000	0.0219	0.0663	0.0137	0.7719	1.0000	0.9736	1.0000	0.0000	0.0000	0.0001	0.8897	1.0000		0.9997	0.9974	0.9796
Wet	Bottom	Pellet	{16}	0.0000	0.0000	0.0365	0.1372	0.0190	0.9757	1.0000	0.9999	1.0000	0.0000	0.0000	0.0001	0.9999	1.0000	0.9997		1.0000	1.0000
Wet	Bottom	Trash fish	{17}	0.0000	0.0000	0.0294	0.1104	0.0156	0.9516	1.0000	0.9994	1.0000	0.0000	0.0000	0.0001	0.9983	1.0000	0.9974	1.0000		1.0000
Wet	Bottom	Outside cages	{18}	0.0000	0.0000	0.0229	0.0849	0.0124	0.9047	1.0000	0.9959	1.0000	0.0000	0.0000	0.0001	0.9563	1.0000	0.9796	1.0000	1.0000	

e. *Enteromorpha clathrata*

ANOVA results

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Season	1	1078.5397	1143	33.6337	32.0672	0.0000
Depth	2	1573.6056	1143	33.6337	46.7866	0.0000
Feed	2	251.5427	1143	33.6337	7.4789	0.0006
Week	7	59.5081	1143	33.6337	1.7693	0.0898
Season x Depth	2	848.0050	1143	33.6337	25.2130	0.0000
Season x Feed	2	145.5363	1143	33.6337	4.3271	0.0134
Depth x Feed	4	205.9486	1143	33.6337	6.1233	0.0001
Season x Week	7	71.8668	1143	33.6337	2.1367	0.0374
Depth x Week	14	48.8432	1143	33.6337	1.4522	0.1222
Feed x Week	14	30.8286	1143	33.6337	0.9166	0.5401
Season x Depth x Feed	4	113.2612	1143	33.6337	3.3675	0.0095
Season x Depth x Week	14	50.7333	1143	33.6337	1.5084	0.1008

Season x Feed x Week	14	53.0917	1143	33.6337	1.5785	0.0786
Depth x Feed x Week	28	28.9603	1143	33.6337	0.8611	0.6747
Season x Depth x Feed x Week	28	48.8804	1143	33.6337	1.4533	0.0604

Post hoc test results (Student – Newman – Keuls test)

				{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}	{17}	{18}
Season	Depth	Feed		11.25	13.27	2.82	1.54	1.12	0.11	1.54	0.22	0.11	1.99	2.30	0.32	0.00	0.00	0.00	0.00	0.00	0.00
Dry	Upper	Pellet	{1}		0.1631	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Dry	Upper	Trash fish	{2}	0.1631		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Dry	Upper	Outside cages	{3}	0.0000	0.0000		0.7081	0.7029	0.6959	0.9309	0.6249	0.9146	0.7139	0.7980	0.6445	0.8946	0.8703	0.8407	0.8048	0.7617	0.7100
Dry	Middle	Pellet	{4}	0.0000	0.0000	0.7081		0.8324	0.9724	1.0000	0.9266	0.9999	0.7617	0.6675	0.8895	0.9998	0.9996	0.9989	0.9973	0.9933	0.9833
Dry	Middle	Trash fish	{5}	0.0000	0.0000	0.7029	0.8324		0.9774	1.0000	0.9133	1.0000	0.7971	0.7974	0.8031	1.0000	0.9999	0.9998	0.9991	0.9970	0.9895
Dry	Middle	Outside cages	{6}	0.0000	0.0000	0.6959	0.9724	0.9774		1.0000	1.0000	1.0000	0.8427	0.9020	0.9866	1.0000	1.0000	1.0000	0.9999	0.9972	0.9433
Dry	Bottom	Pellet	{7}	0.0000	0.0000	0.9309	1.0000	1.0000	1.0000		1.0000	1.0000	0.9882	0.9979	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Dry	Bottom	Trash fish	{8}	0.0000	0.0000	0.6249	0.9266	0.9133	1.0000	1.0000		1.0000	0.7739	0.8334	0.8757	1.0000	1.0000	1.0000	1.0000	0.9999	0.9972
Dry	Bottom	Outside cages	{9}	0.0000	0.0000	0.9146	0.9999	1.0000	1.0000	1.0000	1.0000		0.9831	0.9964	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Wet	Upper	Pellet	{10}	0.0000	0.0000	0.7139	0.7617	0.7971	0.8427	0.9882	0.7739	0.9831		0.7846	0.7720	0.9758	0.9654	0.9508	0.9302	0.9015	0.8615
Wet	Upper	Trash fish	{11}	0.0000	0.0000	0.7980	0.6675	0.7974	0.9020	0.9979	0.8334	0.9964	0.7846		0.8098	0.9940	0.9899	0.9830	0.9717	0.9531	0.9228
Wet	Upper	Outside cages	{12}	0.0000	0.0000	0.6445	0.8895	0.8031	0.9866	1.0000	0.8757	1.0000	0.7720	0.8098		1.0000	1.0000	1.0000	0.9999	0.9994	0.9959
Wet	Middle	Pellet	{13}	0.0000	0.0000	0.8946	0.9998	1.0000	1.0000	1.0000	1.0000	1.0000	0.9758	0.9940	1.0000		1.0000	1.0000	1.0000	1.0000	1.0000
Wet	Middle	Trash fish	{14}	0.0000	0.0000	0.8703	0.9996	0.9999	1.0000	1.0000	1.0000	1.0000	0.9654	0.9899	1.0000	1.0000		1.0000	1.0000	1.0000	1.0000
Wet	Middle	Outside cages	{15}	0.0000	0.0000	0.8407	0.9989	0.9998	1.0000	1.0000	1.0000	1.0000	0.9508	0.9830	1.0000	1.0000	1.0000		1.0000	1.0000	1.0000
Wet	Bottom	Pellet	{16}	0.0000	0.0000	0.8048	0.9973	0.9991	0.9999	1.0000	1.0000	1.0000	0.9302	0.9717	0.9999	1.0000	1.0000	1.0000		1.0000	1.0000
Wet	Bottom	Trash fish	{17}	0.0000	0.0000	0.7617	0.9933	0.9970	0.9972	1.0000	0.9999	1.0000	0.9015	0.9531	0.9994	1.0000	1.0000	1.0000	1.0000		1.0000
Wet	Bottom	Outside cages	{18}	0.0000	0.0000	0.7100	0.9833	0.9895	0.9433	1.0000	0.9972	1.0000	0.8615	0.9228	0.9959	1.0000	1.0000	1.0000	1.0000	1.0000	

f. *Xenostrobus mangle*

ANOVA results

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Season	1	677.5722	1143	13.8955	48.7620	0.0000
Depth	2	470.5930	1143	13.8955	33.8666	0.0000

Feed	2	204.3608	1143	13.8955	14.7070	0.0000
Week	7	161.7103	1143	13.8955	11.6376	0.0000
Season x Depth	2	131.9686	1143	13.8955	9.4972	0.0001
Season x Feed	2	586.8288	1143	13.8955	42.2316	0.0000
Depth x Feed	4	37.6867	1143	13.8955	2.7122	0.0288
Season x Week	7	148.6467	1143	13.8955	10.6975	0.0000
Depth x Week	14	94.6500	1143	13.8955	6.8116	0.0000
Feed x Week	14	63.8797	1143	13.8955	4.5972	0.0000
Season x Depth x Feed	4	175.4164	1143	13.8955	12.6240	0.0000
Season x Depth x Week	14	56.6312	1143	13.8955	4.0755	0.0000
Season x Feed x Week	14	69.8611	1143	13.8955	5.0276	0.0000
Depth x Feed x Week	28	32.6724	1143	13.8955	2.3513	0.0001
Season x Depth x Feed x Week	28	43.1181	1143	13.8955	3.1030	0.0000

Post hoc test results (Student – Newman – Keuls test)

				{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}	{17}	{18}
Season	Depth	Feed		0.19	0.00	5.45	0.29	0.46	0.00	0.29	0.41	0.00	12.12	5.68	0.86	9.21	3.51	0.00	3.08	0.52	0.00
Dry	Upper	Pellet	{1}		0.9996	0.0000	1.0000	1.0000	0.9971	0.9998	1.0000	1.0000	0.0000	0.0000	0.9950	0.0000	0.2296	0.9776	0.9306	1.0000	0.8394
Dry	Upper	Trash fish	{2}	0.9996		0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.9984	0.0000	0.2358	1.0000	0.9551	1.0000	1.0000
Dry	Upper	Outside cages	{3}	0.0000	0.0000		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9353	0.0002	0.5279	0.0327	0.0000	0.0009	0.0000	0.0000
Dry	Middle	Pellet	{4}	1.0000	1.0000	0.0000		0.9996	0.9996	0.9960	1.0000	0.9999	0.0000	0.0000	0.9886	0.0000	0.1972	0.9971	0.8978	0.9997	0.9776
Dry	Middle	Trash fish	{5}	1.0000	1.0000	0.0000	0.9996		1.0000	0.9871	0.9944	0.9601	0.0000	0.0000	0.9126	0.0000	0.1320	0.9999	0.7500	0.9810	0.9997
Dry	Middle	Outside cages	{6}	0.9971	1.0000	0.0000	0.9996	1.0000		1.0000	1.0000	1.0000	0.0000	0.0000	0.9970	0.0000	0.2127	1.0000	0.9393	1.0000	1.0000
Dry	Bottom	Pellet	{7}	0.9998	1.0000	0.0000	0.9960	0.9871	1.0000		0.9321	0.9976	0.0000	0.0001	0.9619	0.0000	0.1629	0.9997	0.8279	0.9971	0.9985
Dry	Bottom	Trash fish	{8}	1.0000	1.0000	0.0000	1.0000	0.9944	1.0000	0.9321		0.9988	0.0000	0.0000	0.9741	0.0000	0.1651	0.9996	0.8505	0.9985	0.9971
Dry	Bottom	Outside cages	{9}	1.0000	1.0000	0.0000	0.9999	0.9601	1.0000	0.9976	0.9988		0.0000	0.0000	0.8155	0.0000	0.1091	1.0000	0.6569	0.8915	0.9998
Wet	Upper	Pellet	{10}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Wet	Upper	Trash fish	{11}	0.0000	0.0000	0.9353	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000		0.0001	0.7562	0.0153	0.0000	0.0007	0.0000	0.0000
Wet	Upper	Outside cages	{12}	0.9950	0.9984	0.0002	0.9886	0.9126	0.9970	0.9619	0.9741	0.8155	0.0000	0.0001		0.0000	0.1646	0.9942	0.5864	0.6369	0.9891
Wet	Middle	Pellet	{13}	0.0000	0.0000	0.5279	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.7562	0.0000		0.0092	0.0000	0.0001	0.0000	0.0000
Wet	Middle	Trash fish	{14}	0.2296	0.2358	0.0327	0.1972	0.1320	0.2127	0.1629	0.1651	0.1091	0.0000	0.0153	0.1646	0.0092		0.1896	0.2038	0.1010	0.1666
Wet	Middle	Outside cages	{15}	0.9776	1.0000	0.0000	0.9971	0.9999	1.0000	0.9997	0.9996	1.0000	0.0000	0.0000	0.9942	0.0000	0.1896		0.9182	0.9999	1.0000
Wet	Bottom	Pellet	{16}	0.9306	0.9551	0.0009	0.8978	0.7500	0.9393	0.8279	0.8505	0.6569	0.0000	0.0007	0.5864	0.0001	0.2038	0.9182		0.5667	0.8901

Wet	Bottom	Trash fish	{17}	1.0000	1.0000	0.0000	0.9997	0.9810	1.0000	0.9971	0.9985	0.8915	0.0000	0.0000	0.6369	0.0000	0.1010	0.9999	0.5667		0.9997
Wet	Bottom	Outside cages	{18}	0.8394	1.0000	0.0000	0.9776	0.9997	1.0000	0.9985	0.9971	0.9998	0.0000	0.0000	0.9891	0.0000	0.1666	1.0000	0.8901	0.9997	

g. *Lyngbya* sp.

ANOVA results

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Season	1	4970.3823	1143	38.1529	130.2754	0.0000
Depth	2	712.1478	1143	38.1529	18.6656	0.0000
Feed	2	1898.2448	1143	38.1529	49.7536	0.0000
Week	7	715.2299	1143	38.1529	18.7464	0.0000
Season x Depth	2	668.5034	1143	38.1529	17.5217	0.0000
Season x Feed	2	1906.7750	1143	38.1529	49.9772	0.0000
Depth x Feed	4	374.0417	1143	38.1529	9.8038	0.0000
Season x Week	7	725.9651	1143	38.1529	19.0278	0.0000
Depth x Week	14	74.5393	1143	38.1529	1.9537	0.0183
Feed x Week	14	1168.8143	1143	38.1529	30.6350	0.0000
Season x Depth x Feed	4	384.5043	1143	38.1529	10.0780	0.0000
Season x Depth x Week	14	74.4311	1143	38.1529	1.9509	0.0185
Season x Feed x Week	14	1170.9938	1143	38.1529	30.6921	0.0000
Depth x Feed x Week	28	115.9772	1143	38.1529	3.0398	0.0000
Season x Depth x Feed x Week	28	117.7770	1143	38.1529	3.0870	0.0000

Post hoc test results (Student – Newman – Keuls test)

				{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}	{17}	{18}
Season	Depth	Feed		7.59	0.12	25.79	6.16	0.00	8.45	6.16	0.00	8.45	0.36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dry	Upper	Pellet	{1}		0.0000	0.0000	0.0615	0.0002	0.7777	0.0995	0.0001	0.5486	0.0000	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0000
Dry	Upper	Trash fish	{2}	0.0000		0.0000	0.0577	1.0000	0.0000	0.0350	1.0000	0.0003	0.8921	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9975	0.9468
Dry	Upper	Outside cages	{3}	0.0000	0.0000		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Dry	Middle	Pellet	{4}	0.0615	0.0577	0.0000		0.3523	0.0536	0.9735	0.3216	0.0968	0.0457	0.2902	0.2584	0.2264	0.1943	0.1626	0.1316	0.1018	0.0738
Dry	Middle	Trash fish	{5}	0.0002	1.0000	0.0000	0.3523		0.0001	0.3426	1.0000	0.0021	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Dry	Middle	Outside cages	{6}	0.7777	0.0000	0.0000	0.0536	0.0001		0.0748	0.0001	0.6515	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Dry	Bottom	Pellet	{7}	0.0995	0.0350	0.0000	0.9735	0.3426	0.0748		0.3099	0.2076	0.0190	0.2766	0.2429	0.2091	0.1754	0.1424	0.1104	0.0803	0.0528
Dry	Bottom	Trash fish	{8}	0.0001	1.0000	0.0000	0.3216	1.0000	0.0001	0.3099		0.0018	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Dry	Bottom	Outside cages	{9}	0.5486	0.0003	0.0000	0.0968	0.0021	0.6515	0.2076	0.0018		0.0003	0.0016	0.0014	0.0011	0.0009	0.0008	0.0006	0.0005	0.0003
Wet	Upper	Pellet	{10}	0.0000	0.8921	0.0000	0.0457	1.0000	0.0000	0.0190	1.0000	0.0003		1.0000	1.0000	1.0000	1.0000	1.0000	0.9996	0.9971	0.9777
Wet	Upper	Trash fish	{11}	0.0001	1.0000	0.0000	0.2902	1.0000	0.0001	0.2766	1.0000	0.0016	1.0000		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Wet	Upper	Outside cages	{12}	0.0001	1.0000	0.0000	0.2584	1.0000	0.0000	0.2429	1.0000	0.0014	1.0000	1.0000		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Wet	Middle	Pellet	{13}	0.0001	1.0000	0.0000	0.2264	1.0000	0.0000	0.2091	1.0000	0.0011	1.0000	1.0000	1.0000		1.0000	1.0000	1.0000	1.0000	1.0000
Wet	Middle	Trash fish	{14}	0.0001	1.0000	0.0000	0.1943	1.0000	0.0000	0.1754	1.0000	0.0009	1.0000	1.0000	1.0000	1.0000		1.0000	1.0000	1.0000	1.0000
Wet	Middle	Outside cages	{15}	0.0001	1.0000	0.0000	0.1626	1.0000	0.0000	0.1424	1.0000	0.0008	1.0000	1.0000	1.0000	1.0000	1.0000		1.0000	1.0000	1.0000
Wet	Bottom	Pellet	{16}	0.0001	0.9999	0.0000	0.1316	1.0000	0.0000	0.1104	1.0000	0.0006	0.9996	1.0000	1.0000	1.0000	1.0000	1.0000		1.0000	1.0000
Wet	Bottom	Trash fish	{17}	0.0001	0.9975	0.0000	0.1018	1.0000	0.0000	0.0803	1.0000	0.0005	0.9971	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000		1.0000
Wet	Bottom	Outside cages	{18}	0.0000	0.9468	0.0000	0.0738	1.0000	0.0000	0.0528	1.0000	0.0003	0.9777	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	

APPENDIX 5

Summary of 3-Way ANOVA and *Post Hoc* Test Results on the Effects of Season (Dry, Wet), Fish Feed (P = Pellet, T = Trash-fish, O = Outside Cages) and Immersion Time (Wk 1, 2, 3…,8) on Abundance of Non-sessile Biofouling Species at Jaha

Appendix 5

Summary of 3-way ANOVA and *Post Hoc* Test Results on the Effects of Season (Dry, Wet), Fish Feed (P = Pellet, T = Trash-Fish, O = Outside Cages) and Immersion Time (Wk 1, 2, 3…,8) on Abundance of Non-sessile Biofouling Species at Jaha

a. Total Amphipods

ANOVA results

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Season	1	50526392.0	94	154831.3281	326.3318	0.0000
Feed	2	27685886.0	94	154831.3281	178.8132	0.0000
Week	7	2203147.5	94	154831.3281	14.2293	0.0000
Season x Feed	2	9879319.0	94	154831.3281	63.8070	0.0000
Season x Week	7	972017.5	94	154831.3281	6.2779	0.0000
Feed x Week	14	593762.8	94	154831.3281	3.8349	0.0000
Season x Feed x Week	14	235974.4	94	154831.3281	1.5241	0.1175

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}
Season	Feed		2500.32	2348.38	323.66	705.23	701.23	168.02
Dry	Pellet	{1}		0.2875	0.0001	0.0001	0.0001	0.0001
Dry	Trash fish	{2}	0.2875		0.0001	0.0001	0.0001	0.0001
Dry	Outside cages	{3}	0.0001	0.0001		0.0036	0.0015	0.1772
Wet	Pellet	{4}	0.0001	0.0001	0.0036		0.9723	0.0002
Wet	Trash fish	{5}	0.0001	0.0001	0.0015	0.9723		0.0001
Wet	Outside cages	{6}	0.0001	0.0001	0.1772	0.0002	0.0001	

b. *Gammaropsis sp.*

ANOVA results

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Season	1	14130612.00	94	45075.2969	313.4891	0.0000
Feed	2	8513995.00	94	45075.2969	188.8838	0.0000
Week	7	1649287.13	94	45075.2969	36.5896	0.0000
Season x Feed	2	2205649.00	94	45075.2969	48.9325	0.0000
Season x Week	7	1925756.13	94	45075.2969	42.7231	0.0000
Feed x Week	14	399191.47	94	45075.2969	8.8561	0.0000
Season x Feed x Week	14	256272.44	94	45075.2969	5.6854	0.0000

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}
Season	Feed		1377.25	1490.33	298.99	529.99	512.78	158.73
Dry	Pellet	{1}		0.2849	0.0001	0.0001	0.0001	0.0001
Dry	Trash fish	{2}	0.2849		0.0001	0.0001	0.0001	0.0001
Dry	Outside cages	{3}	0.0001	0.0001		0.0010	0.0009	0.0256
Wet	Pellet	{4}	0.0001	0.0001	0.0010		0.7812	0.0001
Wet	Trash fish	{5}	0.0001	0.0001	0.0009	0.7812		0.0001
Wet	Outside cages	{6}	0.0001	0.0001	0.0256	0.0001	0.0001	

c. *Photis sp.*

ANOVA results

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Season	1	10142267.00	94	88160.5391	115.0432	0.0000
Feed	2	5079269.00	94	88160.5391	57.6139	0.0000
Week	7	2029632.38	94	88160.5391	23.0220	0.0000
Season x Feed	2	2540868.75	94	88160.5391	28.8209	0.0000
Season x Week	7	813518.13	94	88160.5391	9.2277	0.0000
Feed x Week	14	515041.06	94	88160.5391	5.8421	0.0000
Season x Feed x Week	14	223169.31	94	88160.5391	2.5314	0.0041

Post hoc test results (Student – Newman – Keuls test)

Season	Feed		{1}	{2}	{3}	{4}	{5}	{6}
			1044.79	840.58	19.34	170.94	178.03	5.93
Dry	Pellet	{1}		0.1392	0.0001	0.0001	0.0001	0.0001
Dry	Trash fish	{2}	0.1392		0.0001	0.0001	0.0001	0.0001
Dry	Outside cages	{3}	0.0001	0.0001		0.0826	0.1631	0.8770
Wet	Pellet	{4}	0.0001	0.0001	0.0826		0.9348	0.1414
Wet	Trash fish	{5}	0.0001	0.0001	0.1631	0.9348		0.1981
Wet	Outside cages	{6}	0.0001	0.0001	0.8770	0.1414	0.1981	

d. *Leptognathia* sp.

ANOVA results

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Season	1	25644.67	94	413.1381	62.0729	0.0000
Feed	2	52260.28	94	413.1381	126.4959	0.0000
Week	7	7261.31	94	413.1381	17.5760	0.0000
Season x Feed	2	4665.21	94	413.1381	11.2921	0.0000
Season x Week	7	5785.33	94	413.1381	14.0034	0.0000
Feed x Week	14	1655.29	94	413.1381	4.0066	0.0000
Season x Feed x Week	14	1579.03	94	413.1381	3.8220	0.0000

Post hoc test results (Student – Newman – Keuls test)

Season	Feed		{1}	{2}	{3}	{4}	{5}	{6}
			91.00	78.67	10.25	54.12	30.80	5.97
Dry	Pellet	{1}		0.0038	0.0001	0.0001	0.0001	0.0001
Dry	Trash fish	{2}	0.0038		0.0001	0.0017	0.0001	0.0001
Dry	Outside cages	{3}	0.0001	0.0001		0.0001	0.0003	0.4717
Wet	Pellet	{4}	0.0001	0.0017	0.0001		0.0006	0.0001
Wet	Trash fish	{5}	0.0001	0.0001	0.0003	0.0006		0.0001
Wet	Outside cages	{6}	0.0001	0.0001	0.4717	0.0001	0.0001	

e. Nematodes (undetermined species)

ANOVA Result

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Season	1	19497.2930	94	1573.8080	12.3886	0.0007
Feed	2	950.9850	94	1573.8080	0.6043	0.5486
Week	7	28320.7656	94	1573.8080	17.9951	0.0000
Season x Feed	2	16881.5918	94	1573.8080	10.7266	0.0001
Season x Week	7	12290.5557	94	1573.8080	7.8094	0.0000

Feed x Week	14	1377.1838	94	1573.8080	0.8751	0.5876
Season x Feed x Week	14	2197.0334	94	1573.8080	1.3960	0.1705

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}
Season	Feed		41.99	44.56	82.20	84.76	93.02	62.23
Dry	Pellet	{1}		0.8250	0.0042	0.0033	0.0005	0.1912
Dry	Trash fish	{2}	0.8250		0.0045	0.0042	0.0007	0.1292
Dry	Outside cages	{3}	0.0042	0.0045		0.8249	0.6178	0.0869
Wet	Pellet	{4}	0.0033	0.0042	0.8249		0.4759	0.1298
Wet	Trash fish	{5}	0.0005	0.0007	0.6178	0.4759		0.0438
Wet	Outside cages	{6}	0.1912	0.1292	0.0869	0.1298	0.0438	

f. Total Copepods

ANOVA results

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Season	1	29501.0039	94	354.7032	83.1709	0.0000
Feed	2	30163.3438	94	354.7032	85.0382	0.0000
Week	7	3795.8022	94	354.7032	10.7013	0.0000
Season x Feed	2	6239.6240	94	354.7032	17.5911	0.0000
Season x Week	7	4498.5132	94	354.7032	12.6825	0.0000
Feed x Week	14	2256.5815	94	354.7032	6.3619	0.0000
Season x Feed x Week	14	1068.5343	94	354.7032	3.0125	0.0007

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}
Season	Feed		14.79	13.29	38.05	35.23	24.98	93.56
Dry	Pellet	{1}		0.7858	0.0004	0.0010	0.0659	0.0001
Dry	Trash fish	{2}	0.7858		0.0003	0.0008	0.0885	0.0001
Dry	Outside cages	{3}	0.0004	0.0003		0.6086	0.0496	0.0001
Wet	Pellet	{4}	0.0010	0.0008	0.6086		0.0645	0.0001
Wet	Trash fish	{5}	0.0659	0.0885	0.0496	0.0645		0.0001
Wet	Outside cages	{6}	0.0001	0.0001	0.0001	0.0001	0.0001	

APPENDIX 6

Summary of 3-Way ANOVA and *Post Hoc* Test Results on the Effects of Season (Dry, Wet), Fish Feed (P = Pellet, T = Trash-Fish, O = Outside Cages) and Immersion Time (Wk 1, 2, 3…,8) on Biomass of Sessile and Non-Sessile Biofouling Organisms at Jaha

Appendix 6

Summary of 3-way ANOVA and *Post Hoc* Test Results on the Effects of Season (Dry, Wet), Fish Feed (P = Pellet, T = Trash-Fish, O = Outside Cages) and Immersion Time (Wk 1, 2, 3…,8) on Biomass of Sessile and Non-sessile Biofouling Organisms at Jaha

a. Sessile biofouling

ANOVA results

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Season	1	963652.9375	94	5544.2300	173.8119	0.0000
Feed	2	525564.6250	94	5544.2300	94.7949	0.0000
Week	7	185117.3906	94	5544.2300	33.3892	0.0000
Season x Feed	2	222876.4375	94	5544.2300	40.1997	0.0000
Season x Week	7	76735.5078	94	5544.2300	13.8406	0.0000
Feed x Week	14	21559.4922	94	5544.2300	3.8886	0.0000
Season x Feed x Week	14	18157.3516	94	5544.2300	3.2750	0.0003

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}
Season	Feed		505.03	445.29	183.40	213.05	257.86	161.86
Dry	Pellet	{1}		0.0071	0.0001	0.0001	0.0001	0.0001
Dry	Trash fish	{2}	0.0071		0.0001	0.0001	0.0001	0.0001
Dry	Outside cages	{3}	0.0001	0.0001		0.1743	0.0026	0.3226
Wet	Pellet	{4}	0.0001	0.0001	0.1743		0.0414	0.0522
Wet	Trash fish	{5}	0.0001	0.0001	0.0026	0.0414		0.0003
Wet	Outside cages	{6}	0.0001	0.0001	0.3226	0.0522	0.0003	

b. Non-sessile biofouling

ANOVA results

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Season	1	19458.0195	94	82.3375	236.3204	0.0000
Feed	2	16847.0098	94	82.3375	204.6093	0.0000
Week	7	2249.3694	94	82.3375	27.3189	0.0000
Season x Feed	2	4341.7622	94	82.3375	52.7313	0.0000
Season x Week	7	643.5933	94	82.3375	7.8165	0.0000
Feed x Week	14	539.8336	94	82.3375	6.5564	0.0000
Season x Feed x Week	14	181.6650	94	82.3375	2.2063	0.0128

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}
Season	Feed		59.96	56.14	8.94	22.61	24.17	7.07
Dry	Pellet	{1}		0.1514	0.0001	0.0001	0.0001	0.0001
Dry	Trash fish	{2}	0.1514		0.0001	0.0001	0.0001	0.0001
Dry	Outside cages	{3}	0.0001	0.0001		0.0001	0.0001	0.4818
Wet	Pellet	{4}	0.0001	0.0001	0.0001		0.5568	0.0001
Wet	Trash fish	{5}	0.0001	0.0001	0.0001	0.5568		0.0001
Wet	Outside cages	{6}	0.0001	0.0001	0.4818	0.0001	0.0001	

APPENDIX 7

Effects of Water Flow Velocity and Fish Culture on Net Biofouling in Fish Cages

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Effects of water flow velocity and fish culture on net biofouling in fish cages

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Abstract

The effects of water flow, fish feed and cage position on net biofouling was examined in a floating cage fish farm. Fouling of 16 mm mesh net panels suspended inside and outside net cages and exposed to different treatments were monitored weekly until net apertures were completely occluded by the fouling organisms (8 weeks). Results indicate a dramatic reduction in water flow velocity throughout the fish farm due to the cage units themselves and net biofouling. The reduced water flow ($< 10\ cm\ s^{-1}$) inside net cages promoted rapid net biofouling, while rapid water flow outside the net cages ($> 25\ cm\ s^{-1}$) kept the net fouling organisms at bay. Although fish rearing in net cages with inputs of commercial pellet feed increased sessile biofouling (222% higher than outside the net cages) and non-sessile biofouling (570% higher), the type of fish feed used did not significantly affect biofouling development. The study recommends that the geometry of serially arranged net cages, as commonly deployed in tropical tidal estuaries, be reconfigured to improve flow through in order to minimize the impact of fouling.

Keywords: net biofouling, water flow velocity, fish feed, floating fish-cages, tropical estuary, aquaculture Malaysia

Introduction

Marine biofouling of floating net cages continuously plagues fish farmers. The fouled net has to be regularly removed and cleaned of its burden. In tropical fish farms, the method used is to first dry the fouled net for several days under the hot sun, then break up the encrusting shells and finally clean the net using a high-pressure water pump. Net cleaning not only incurs energy costs, but is labour intensive and damages the net over the long term (Hodson, Lewis & Burke 1997). However, failure to conduct net changes after heavy fouling often results in problems attributable to poor water quality and net strain (e.g. Phillippi, O'Conner, Lewis & Kim 2001; Swift, Fredriksson, Unrein, Fullterton, Patursson & Baldwin 2006). The consequences could be fish asphyxiation and loss of cultured fish due to net ruptures. The operation of changing cage nets often injures and stresses the cultured fish, which may result in mortality or reduced growth of fish (Hodson *et al.* 1997).

Various factors are thought to influence biofouling development in aquaculture. It has been suggested that fish farm water is rather conducive to rapid biofouling due to the high level of nutrient and organic loadings associated with fish rearing (Ruokolahti 1988; Costa-Pierce & Bridger 2002; Cook, Black, Sayer, Cromey, Angel, Spanier, Tsemel, Katz, Eden, Karakassis, Tsapakis, Apostolaki & Malej 2006). Feed wastage is an important source of nutrient loading and organic matter (Seymour & Bergheim 1991). The amount of feed wastage depends on feed type and composition, fish stocking density, feeding method and feeding rates (Beveridge, Phillips & Clarke 1991).

The use of a low-quality feed such as trash fish is known to produce a higher amount of food wastage including uneaten feed and solid particles. For exam-

ple, APEC/NACA/BOBP/GOI (2002) reported that solid wastes produced from the use of trash fish feed was 40% higher than the use of pellet feed. The amount of supplied feed and its digestibility are among the other important factors that influence waste output from fish culture. With poor feed quality, the amount of food that will end up as faeces will increase due to a poor food conversion ratio (Cho, Hynes, Wood & Yoshida 1994). Feed wastage and faecal matter can be reduced if a high-quality feed with high digestibility and an optimal protein to energy ratio is used (Wu 1995). Thus, good-quality feed, which is necessary for successful aquaculture, has the additional benefit of less feed wastage and possibly less biofouling.

The hydrographic condition and ambient water quality combine with feed wastage to influence biofouling. Water current and tidal regimes are among the important factors known to influence the succession and colonization rates of biofouling organisms (Baynes & Szmant 1989; Yan, Yan, Dong, Wang, Yan & Liang 2006). For example, water velocity influences the differential supply and attachment of larvae on the surface (Abelson & Denny 1997) as well as epifaunal growth (Leichter & Witman 1997), while tidal currents are known to be efficient conveyors of fouling organisms on coastal installations (Yan *et al.* 2006). Other parameters such as temperature, salinity, light and turbidity have been suggested to play a role in the development of biofouling assemblages (see Bombace, Fabi, Fiorentini & Speranza 1994; Underwood & Keough 2001; Qvarfordt, Kautsky & Malm 2006). Water quality parameters, however, vary with fish farm location and possibly even the net cage position within the farm.

The floating net cage culture industry in Malaysia is a relatively recent development with large-scale farming in marine waters starting in the 1980s. It is now the fastest growing sector in the aquaculture industry, with 82 800 net cages (104 ha), which produced 15 122 tonnes in 2007 (Anonymous 2007). As the number of marine cage net farms is expected to increase in line with the country's long-term Aquaculture Development Action Plan to expand the sector at an annual rate of 20%, cage units are expected to crowd limited water space such as in the tidal estuaries and coastal bays. Net biofouling is expected to be an important problem, not only as an operational cost but also as a production liability if reduced fish growth and mortality occur. The common problems faced by cage fish culturists in Malaysia have been reported as hypoxia and fish asphyxiation particularly in the early morning, eutrophication, disruption of water flow, sedimentation, frequent net cleanings and fish diseases (Cheah & Chua 1979; Lai, Kessler & Khoo 1993; Chong, Alongi, Natin, Ooi, Sasekumar & Wong 2004; Madin, Chong & Basri 2009). Probably, the most important contributory factor to these problems is the geometry of the deployed net cages that are set end-to-end, side-by-side and farm-to-farm in a grid-like fashion often stretching across kilometres of surface water. There are, however, a lack of studies on the hydro-engineering aspects of cage culture in tropical settings, such as those pertaining to farm settings, cage configuration and deployment and cage-building material. Moreover, quantitative data on biofouling pertaining to the aquaculture environment are poor and of limited use despite the fact that research on net biofouling began three decades ago (Braithwaite & McEvoy 2005). Q2

This study thus addressed the biofouling problem in the floating cage net culture. It examined how fish culture in net cages altered the flow dynamics which, together with feed inputs, affects biofouling on cage nettings.

Materials and methods

Study site

The study was carried out at a fish farm in the Jaha River estuary, which had only two fish farms with a total of 600 floating net cages. The small estuary comprises one of the many waterways that drain the Matang Mangrove Forest Reserve (MMFR), Malaysia (Fig. 1). The major waterway of the MMFR, the Sangga Besar river, however, has the highest concentration of floating fish farms with nearly 4000 cage units. The floating fish farms in the MMFR mainly culture the giant sea perch (*Lates calcarifer* [Bloch]), golden snapper (*Lutjanus johnii* [Bloch]) and red snapper (*Lutjanus argentimaculatus* [Forsskål]).

The Jaha River estuary is shallow averaging 3 m deep. Water is well mixed and the tides are semi-diurnal with maximum tidal amplitudes of 2.5 m during spring tide and 1.7 m during neap tide. The floating cage net farm was located ca. 20–30 m from the river bank and extended another 30 m to midstream. The farm was permanently positioned with the use of metal and concrete anchors at both ends and kept afloat through the use of empty polythene barrels. There were 258–288 interconnected net cages, which were regularly used for fish rearing in the farm which contained 300 net cage frames. Net cages, each with a Q3

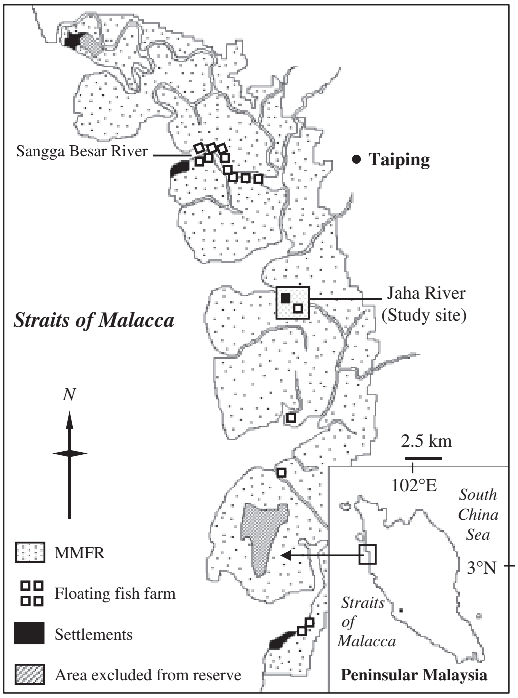

Figure 1 Location of study site at Jaha River estuary (boxed) in the Matang Mangrove Forest Reserve (MMFR), Perak, Peninsular Malaysia. Filled square indicates an experimental farm.

dimension of 2.5 m (*L*) × 2.5 m (*W*) × 1.5 m (*D*), were arranged in a serial grid with inter-cage gaps of 0.5 m.

Experimental design and layout

The study was carried out from May to July 2005. In the first experiment, nine unfouled net cages were deployed at the downstream end of the farm to investigate the effects of water flow velocity and two types of pellet feed with different water stability on biofouling development (Fig. 2a). The experimental net cages were set up in triplicates for each of the following four treatments: (1) stocked fish fed commercially produced extruded pellet feed (M1, M2 and M3), (2) stocked fish fed home-made pellet feed (P1, P2 and P3) and (3) no stocked fish and no feed given (N1, N2 and N3). Another treatment was located outside the net cages and was referred to as (4) negative control, i.e. no fish, feed and enclosure (C1, C2 and C3). The C treatments outside the cages were expected to have higher water flow through them as compared with the M, P and N treatments inside the net cages.

The experimental net cages were set up at the downstream end of the farm in order to reduce contamination from upstream cages although fish feedings were carried out during low slack and most feed were consumed within the first 15 min. The limited space for rearing fingerlings only allowed the triplicates to be arranged linearly in triplets (i.e. along main axis of river) at one half of the small farm. Nevertheless, each member of a triplet was assumed to be exposed to similar physical conditions as their counterpart on the other treatment triplet (e.g. N2, P2 and M2). Preliminary current measurements inside similarly arranged net cages had shown that the flow rates were not significantly different among members of a triplet, except at the start of the experiment when the nets were clean but even then the current velocity became rather homogeneous across the farm as biofouling progressed.

The commercially produced extruded feed pellets (Charoen Pokphand Feedmill), of 4 mm in diameter, were composed of 40% protein and 0.4% lipid with a maximum moisture of 12%. The home-made pellets, which had a similar pellet size, were composed of 42.5% crude protein, 12.6% crude lipid and 13.1% ash, with a moisture content of 5.4%. Based on a simple stability test, the commercial feed pellet maintained stability without disintegration for 30 min, whereas the home-made pellet feed (P) disintegrated completely within the same time. The latter also had a high amount of unbound food particles. Both types of feed pellets were of the slow-sinking type with similar main ingredients (i.e. poultry offal meal). Stocked cages were filled with 200 giant sea perch (*Lates calcarifer*) fingerlings (27 ± 4.5 g), which were fed daily at a rate of 3–4% of the total biomass of stocked fish. Q4

A second set of experiments was conducted 4 weeks thereafter to examine the effects of fish feed and net cage position on biofouling development on nets. The net cage position is the location of the cage unit along and across the water channel; it will primarily determine the flow rate. Its layout was based on a Latin Square Design with one and only one treatment replicate on each row and column. Nine, non-fouled net cage units were deployed; three on the upstream end of the farm, three on the downstream end and three between these locations, i.e. mid-position (Fig. 2b). Each block contained three net cages and each net cage received either commercially produced extruded pellet feed (M), trash fish feed (T) or no feed (N). Only M and T cages were each stocked with 100 giant sea perch fingerlings, which were fed

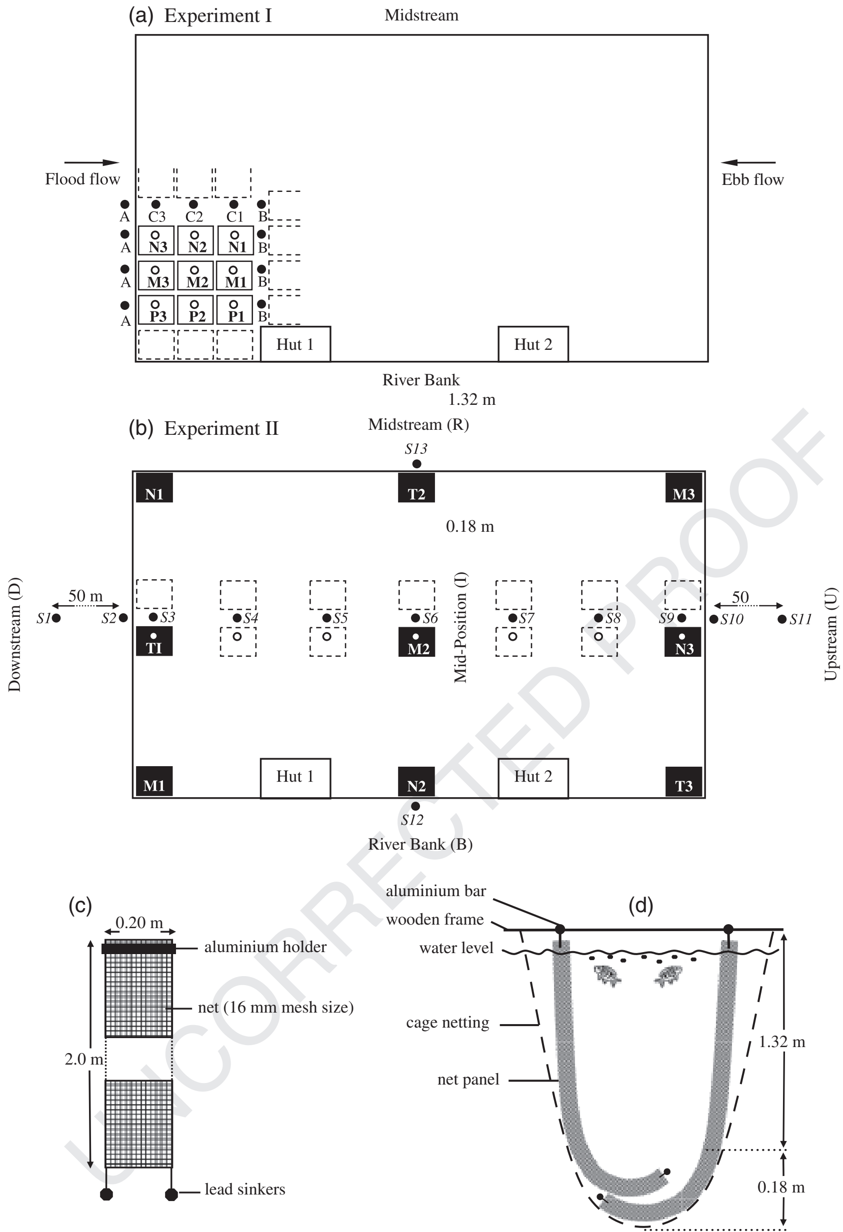

Figure 2 Experimental design and cage layout of study (not to scale). Only experimental net cages, in solid lines, are shown; some non-experimental cages are shown in broken lines; open and filled circles indicate measurements taken inside and outside net cages respectively. (a) Treatments of Experiment I: net cages with stocked fish fed commercially produced extruded pellet feed (M1, M2 and M3); net cages with stocked fish fed home-made pellet feed (P1, P2 and P3); net cages without fish and feed (N1, N2 and N3); and outside the fish-cages without given feed (C1, C2 and C3). (b) Treatments of Experiment II (Latin Square Design): net cages stocked with fish fed with trash fish feed (T1, T2 and T3); and net cages given treatment M and N as above. Physical measurements were made along transect D–I–U from stations S1–S11; S12 at river bank and S13 at midstream. (c) Sketched diagram of single experimental net panel. (d) Cross-sectional view of cage unit showing how two net panels (exaggerated thickness) were deployed with the upper 1.32 m of the net panel vertically positioned, while the lower one-third curved over the tapering net cage bottom.

daily. The design thus examined the effects of fish feed treatment (M, T and N) along the longitudinal (D–I–U) and cross-river (R–I–B) axes of the farm. Trash fish were ground to a sticky pulp containing particulates of very variable sizes. The use of ground trash fish is a common practice of fish feeding in the country.

For both experiments, eight multifilament nylon net panels each of 0.2 m × 2 m dimension (0.4 m^2) were placed inside each experimental net cage (Fig. 2c). The netting mesh size was 16 mm, while filament mean diameter was 1.2 mm. The mesh material, mesh size and filament thickness of net panels used were identical to the nettings of the cage units that enclosed them. The lower end of each net panel was weighted down using lead sinkers to a vertical depth of 2 m with the upper end tied to an aluminium bar held horizontally across the cage frame. The upper part (0–1.32 m) of the net panels was vertical, while the lower part (1.32–2 m) gently curved over to follow the contour of the tapering net cage bottom (Fig. 2d). Four nylon panels each were placed on opposite sides of each cage unit in an alternate fashion to avoid an overlap of their curved sections at the bottom of the cage.

In Experiment I, both P and M panels differed from N and C panels in terms of regular exposure to fish feed and faeces. The difference between N and C net panels was that the latter experienced stronger water flow through them than the former in a flow-reduced environment (inside the net cage). In Experiment II, net panels sited at the mid-position of the farm were expected to experience slower water flow as compared with those on the outer perimeter. Net panel biofouling in both experiments was measured every week for 8 weeks, by removing one net panel from each experimental cage or outside it each week. The removed net panels were immediately immersed in buffered 10% formalin in separate 1 L jars.

Physical and chemical parameters

In Experiment I, water parameters such as salinity, pH, dissolved oxygen (DO) and turbidity were measured fortnightly at the surface (0.5–0.75 m depth) and net cage bottom (1–1.5 m depth), using a Hydrolab DataSonde 4a (Hydrolab, Austin, TX, USA). The water parameters were recorded 2 h before fish feeding and before the experimental net panels were sampled. Water flow through the net cages was determined fortnightly from the downstream position (A) through net cages 3, 2 and 1 to the upstream position (B) during the neap flood flow (see Fig. 2a). The flow rates were measured using a Toho-Dentan electric current meter Model CM-2 (Toho-Dentan, Tokyo, Japan) at a depth of 0.5–0.75 m, from 1.5 h after low slack until 3 h later. Repeated measurements of 20-s average flow velocity were taken three to five times per position during this period. The percentage of flow attenuation on transmission through the series of net cages was determined as follows:

$$\text{Water flow attenuation}\ (\%) = \frac{[(\text{Velocity at position A}) - (\text{Velocity at other position})]}{(\text{Velocity at position A})} \times 100$$

In Experiment II, water flow, pH, temperature, salinity, DO and turbidity were measured fortnightly along the transect (D–I–U) of 13 sampling stations established across the entire farm during the flood and ebb flow at neap tide (see Fig. 2b). Sampling stations S1, S2, S10, S11, S12 and S13 were located outside the farm, whereas stations S3, S4, S5, S6, S7, S8 and S9 were located within the farm with varying distances of 5–7.5 m between adjacent stations. Outside the farm, measurements were taken at both surface (0.5–0.75 m depth) and bottom (2.5–3 m depth) waters. Within the farm, measurements were taken at three positions per station, i.e. surface, inside the net cage (0.5–0.75 m depth); surface, outside the net cage (0.5–0.75 m depth) and bottom, outside the net cage (2.5–3 m depth). All measurements inside the cage were taken at the centre of the cage. Outside the cage, measurements were made at its equivalent position with respect to the direction of main flow. Along the transect, measurements at station commenced from one end to the opposite end of the farm, starting 1.5 h after slack water. Measurements were then repeated in the opposite direction and back-and-forth, until the operation stopped 3 h later. The staying time per station was not more than 3 min and the time for completing measurements along one transect (S1–S11) was 30–40 min.

Laboratory treatment and analysis

In the laboratory, each net panel was gently agitated, removed from its jar and weighed.

The agitation was to remove non-sessile organisms for a later examination. The difference in preserved wet weight before and after the experiment represented the weight of the sessile biofoulers (g per panel). Non-sessile organisms, which had dropped to the bottom of the bottle after agitation, were collected by sieving the entire fluid through a 125 μm mesh Endecott sieve and rinsed quickly with running tap water to remove the fine sediment. The organisms were then placed onto a preweighed wire gauze of the same mesh size and blot-dried before

their combined wet weight was determined (g per panel). They were immediately resuspended in 70% alcohol solution for storage. Sessile and their non-sessile associates were subsequently identified to the lowest taxon possible using a stereo microscope; these results are, however, not fully reported here but in Madin *et al.* (2009).

Computation and statistical analysis

Computed biomass data were subjected to logarithmic $[\log_{10}(x+1)]$ transformation to achieve normality and homogeneity of variance before statistical analysis (Zar 1998). For Experiment I, a two-factor analysis of variance (ANOVA) with equal replication was carried out to investigate the effects of feed treatment (P, M, N and C) and immersion week (1, 2, 3, . . ., 8) on biofouling biomass (g per panel) of sessile and non-sessile organisms. If the ANOVA was significant ($P \leq 0.05$), the Student–Newman–Keuls test was used for multiple comparisons of the means. For Experiment II, the biofouling biomass values among block treatments (feed, longitudinal position, cross-river position) on a 3×3 Latin Square Design were analysed for significant differences each week, for sessile and non-sessile organisms. The tested null hypothesis of no significant difference was rejected at the 5% significance level or if $P < 0.05$. The correlation (r) between biomass of non-sessile and sessile organisms was determined for each treatment. All statistical analyses including for the Latin Square Design were carried out using the STATISTICA version 8 software program.

The mean and standard deviation of the neap tidal velocities recorded from 1.5 h after slack water to 3 h later, during flood tide, were calculated for each week of measurement in Experiment I, while the same were calculated based on measurements from three fortnightly occasions, for both flood and ebb tides in Experiment II. Current velocities recorded about midway between slack tides are considered to be the swiftest within the tidal cycle.

Limitations

The hung net panel was meant to simulate as close as possible the cage unit netting as a substrate for biofouling. It is, however, not exactly identical in contour but the conditions inside the cage unit were the exact conditions for the hung panel. The requirement for weekly monitoring of biofouling biomass including the community structure (in Madin *et al.* 2009), concomitant with fish rearing, necessitates such a methodology, which would allow the random samplings of similar net panels week after week. Thus, we used a completely randomized design and the sampled net panels were assumed to be independent of each other. A repeated measure design, i.e. monitoring of the same panel week after week, would have been statistically more powerful and realistic, but its benefits would have been offset by the repeated disturbance of sampled fauna (e.g. when out of water) and sampling of non-sessile organisms would displace or leave none behind for the next sampling. On the other hand, the completely randomized design has none of these problems except that future population could sometimes be less than the past population due to sampling and uneven growth. However, replications (including stratified samplings for the community analysis) reduced this problem to some extent.

Results

Current flow and other environmental parameters

In Experiment I, water current velocity was reduced by 20% to 90% as water flowed 10 m through the three serial, clean or unfouled fish cages at week 0 (Fig. 3). However, the velocity of the water on encountering the first net cage was reduced by 79% just after 2 weeks of immersion, and subsequently to as high as 91% reduction with further biofouling development. Flow attenuation also further increased to 89% as the water flowed through the serial net cages to position B at week 2. However, the flow attenuation only marginally increased (up to 91%) even at week 8. The results indicated that the physical presence of the net cages themselves drastically obstructed water flow, as for instance, over 75% attenuation was obtained after only passing through one clean net cage unit (i.e. at net cage 2). Outside the net cages and in between the linearly arranged net cages (A–C3–C2–C1–B), the water flowed unimpeded.

The effect of biofouling, which reduced the flow rate by an additional 60% on week 2 and another 10% on week 4, was clearly obvious inside the first net (net cage 3), but not so in net cages further on the leeside where the water flow was so greatly reduced that the measured flow rates among net cage 2, 1 and position B were not significantly different.

Temperature, pH, salinity, DO and turbidity readings were not significantly different among net cages

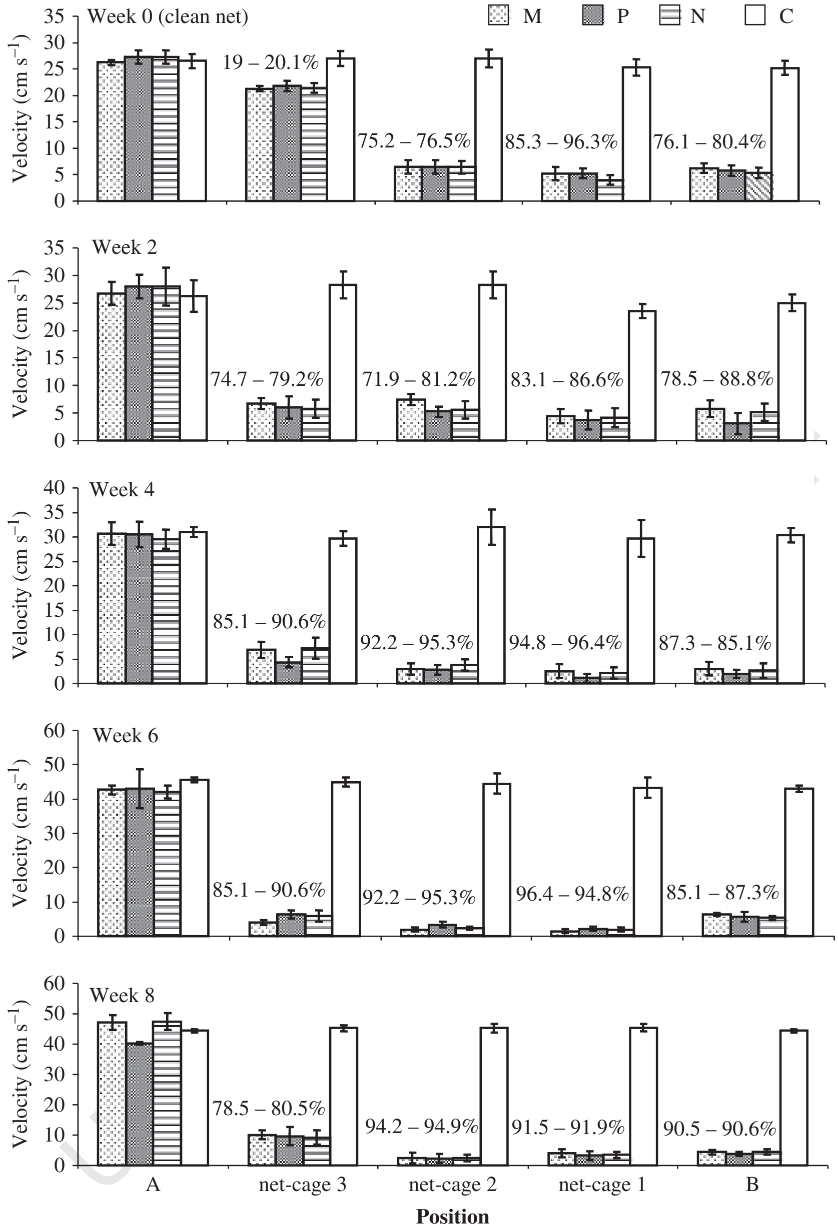

Figure 3 Fortnightly measurements of mean (± SD) of flood tidal velocities (cm s^{-1}) through the serial fish cages (position A through net cages 3, 2, 1 to position B), beginning from week 0 (clean net) to week 8 (Experiment I). Percentage numbers indicate per cent reduction of velocity of incoming current measured at position A. See Fig. 2a for further explanation.

given the different types of treatment, for both ebb and flood water (Table 1). However, DO, salinity and temperature varied significantly between the flood and ebb water.

In Experiment II, the mean surface flood flow velocities recorded along the transect D–I–U inside the farm (Stations S3–S9) were significantly ($P < 0.001$) much lower inside the net cages (4.3 cm s^{-1}) than outside the net cages (30.5 cm s^{-1}). However, the bottom velocity (39.6 cm s^{-1}) outside the net cages but within the farm area was the highest (Fig. 4). This was also true during ebb flow through the farm, where the mean surface velocity inside net cages (3.6 cm s^{-1}) was the lowest, as compared with the

Table 1 Mean values of some environmental parameters recorded in treatment M (stocked fish fed commercially produced extruded pellet feed), P (stocked fish fed home-made pellet feed), N (net cage without fish and feed) and C (no fish, feed and enclosing cage-netting) during the flood and ebb water in a floating fish farm at Jaha River estuary (Experiment I)

	Treatments (mean ± SD)			
	M	P	N	C
Flood pH	7.7 ± 0.2	7.6 ± 0.2	7.7 ± 0.1	7.6 ± 0.2
Temperature (°C)	31.8 ± 0.8	31.7 ± 0.7	31.1 ± 0.4	31.7 ± 0.8
Salinity (ppt)	25.6 ± 1.8	23.3 ± 7.4	26.1 ± 0.8	26.0 ± 1.4
DO (mg L^{-1})	6.5 ± 2.2	5.7 ± 2.5	6.3 ± 0.7	6.2 ± 2.3
Turbidity (NTU)	78.3 ± 28.6	81.1 ± 61.8	75.2 ± 24.5	81.6 ± 36
Ebb pH	7.1 ± 0.1	7.1 ± 0.1	7.2 ± 0.1	7.0 ± 0.1
Temperature (°C)	31.2 ± 0.5	30.8 ± 0.3	30.9 ± 0.3	30.9 ± 0.3
Salinity (ppt)	23.8 ± 0.1	22.2 ± 1.0	21.2 ± 0.1	23.0 ± 1.1
DO (mg L^{-1})	2.9 ± 0.6	2.9 ± 1.6	2.5 ± 0.9	2.3 ± 0.9
Turbidity (NTU)	103.5 ± 16.6	71.0 ± 48.1	87.8 ± 13.7	73.0 ± 23.2

mean surface velocity (20 cm s^{-1}) and bottom velocity (26.3 cm s^{-1}) measured outside the net cages. Although the reduction in flow velocity on meeting the floating net cage farm (i.e. at S3) was very drastic (up to 83%) inside the net cages, there was surprisingly little velocity attenuation as the water flowed 60 m further through the farm.

The various water parameters measured along the transect D–I–U varied significantly with tidal phase (Fig. 5). During flood tide, DO, temperature and pH at all measured positions (SI, SO and BO) markedly decreased from downstream to upstream through the farm, but with no marked changes for salinity and turbidity. Mean surface DO, outside (6.4 mg L^{-1}) and inside (6.3 mg L^{-1}) the net cages were significantly higher than at the bottom (4.6 mg L^{-1}). Turbidity was significantly higher at the bottom water (36.7 NTU) as compared with the surface water whether outside (28 NTU) or inside (28.1 NTU) the net cages. Temperature and salinity did not differ significantly among positions. During ebb tide, pH and turbidity decreased markedly from upstream to downstream but the other parameters (DO, salinity, temperature, turbidity and pH) did not vary spatially. Water turbidity during ebb flow was higher than during flood flow; it, however, decreased sharply from the upstream to downstream end of the farm (S6–S11).

Effects of fish feed on biofouling development (Experiment I)

Sessile macrofouling organisms on the net panels were initially dominated by hydroids (*Plumularia* sp.), but other taxa such as algae (*Polysiphonia* sp., *Enteromorpha clathrata* [Roth] and *Lyngbya* sp.), barnacles (*Balanus amphitrite* [Darwin]), mussels (*Xenostrobus mangle* [Ockelmann]) and anthozoans appeared after 2 weeks; the fouling organisms displayed a clear vertical distribution (Madin *et al.* 2009). Non-sessile organisms included tanaids, nematodes, copepods and polychaetes, but were dominated by amphipods (90%) mainly *Gammaropsis* and *Photis* species.

The main effects of fish rearing, time (weeks) and their interactions were all significant ($P < 0.0001$) on sessile biofouling, explaining 29%, 60% and 7% of its total variability in biomass respectively (Table 2). Fouling biomass (g per panel) was significantly higher inside (P, M and N) than outside (C) the cages, higher in feed-given (P, M) than no-feed (N, C) treatments, but with no significant difference between the two types of feed (P, M). The biofouling biomass increased steadily and significantly with time until week 6 and stabilized thereafter (Fig. 6). For non-sessile fouling, the main effects of feed, time and their interaction were similarly very significant, explaining 42%, 40% and 14% of the total biomass variability (see Table 2). The feed effect among treatments was similar to sessile fouling. The time effect was also significant among weeks, except for the following homogeneous groups (weeks): (1, 2), (4, 5) and (6, 7, 8).

At the end of the eighth week, sessile biofouling on net panels for treatments M, P and N had a mean biomass (g per panel) of 906.1, 932.7 and 691.4 g, respectively, as compared with only 281.6 g for treatment C (see Fig. 6). Mean biomass (g per panel) of non-sessile organisms rapidly increased after the third week, reaching the highest value by the seventh week for treatments M (110.6 g) and P (113.1 g). These values

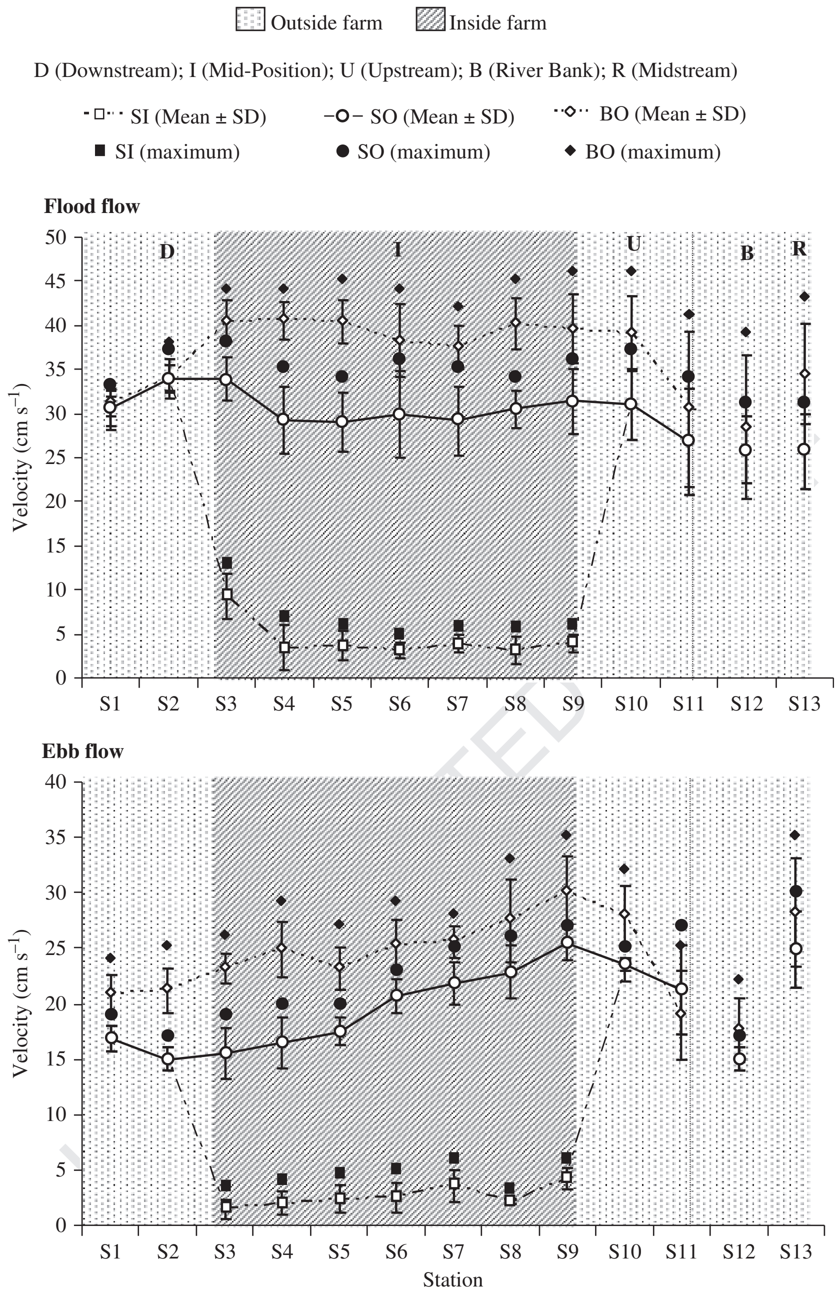

Figure 4 Mean (± SD) and maximum of flood and ebb tidal velocities (cm s^{-1}) recorded along the transect D–I–U (stations S1–S11), S12 at the river bank (B) and S13 at midstream (R) (Experiment II). Measurements were made at the surface, inside net cages (SI); surface, outside net cages (SO) and bottom, outside net cages (BO). See Fig. 2b for further explanation.

were significantly higher than for the treatment N (22.2 g) and C (17 g).

The results indicate that the biomass of non-sessile organisms was significantly correlated to the biomass of sessile organisms inside the M (r = 0.085), P (r = 0.81) and N (r = 0.65) net cages, as well as outside the cages in C (r = 0.60).

Effects of fish feed and net cage position on biofouling development (Experiment II)

The highest biomass (g per panel) of sessile biofoulers on net panels was almost achieved by the sixth week for net cages with fish rearing, i.e. those stocked with fish fed with either commercial feed pellet, M

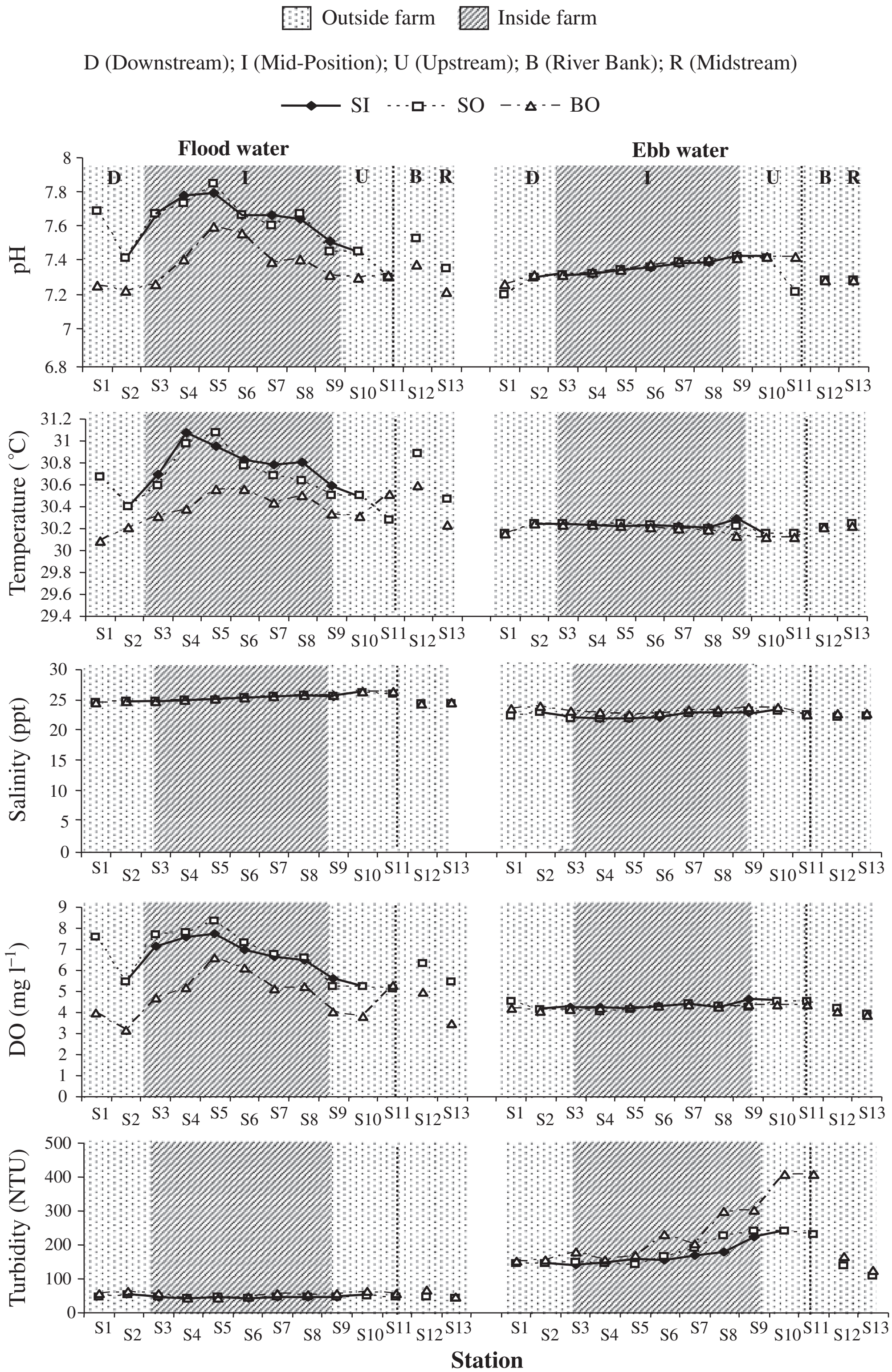

Figure 5 Mean pH, temperature, salinity, dissolved oxygen (DO) and turbidity values recorded along the transect D–I–U (S1–S11), S12 at the river bank (B), and S13 at midstream (R), during flood and ebb tide (Experiment II). Measurements were made at the surface, inside net cages (SI); surface, outside net cages (SO) and bottom, outside net cages (BO).

Table 2 Summary of two-way ANOVA and *post hoc* test results on the effects of fish feed (M = commercial pellet, P = home-made pellet, N = without fish and feed and C = outside the fish cages) and immersion time (week 1, 2, 3, . . ., 8) on the biomass of sessile and non-sessile biofouling, Experiment I

Sessile organisms

ANOVA result

Source of variation	df	SS	*F*	*P*
Feed (A)	3	1.90	148.30	< 0.000001
Immersion time (B)	7	3.99	133.32	< 0.000001
A × B	21	0.49	5.49	< 0.000001
Error	64	0.27		

Post hoc test results*

Feed (A): M (542.9) P (534.7) N (442.8) C (219.7)

Immersion time (B): week 8 (703) week 7 (657.5) week 6 (654.2) week 5 (465.5) week 4 (340) week 3 (265.6) week 2 (215.9) week 1 (178.4)

A × B: Week 1 P (198.6) N (191.1) M (181.5) C (142.5)
Week 2 P (246.9) N (232.6) M (201) C (183.2)
Week 3 M (339.7) P (282.8) N (280.4) C (159.7)
Week 4 P (411.9) M (388) N (370.3) C (190)
Week 5 M (624.1) P (526.2) N (462.8) C (249.1)
Week 6 M (841.1) P (825.7) N (623.7) C (326.2)
Week 7 M (862) P (852.6) N (690.5) C (225.1)
Week 8 P (932.7) M (906.1) N (691.4) C (281.6)

Non-sessile organisms

ANOVA result

Source of variation	df	SS	*F*	*P*
Feed (A)	3	5.47	282.96	< 0.000001
Immersion time (B)	7	5.21	115.44	< 0.000001
A × B	21	1.83	13.49	< 0.000001
Error	64	0.41		

Post hoc test results*

Feed (A): P (59.7) M (55.1) N (16.7) C (13.3)

Immersion time (B): week 7 (65.7) week 8 (56.6) week 6 (49.8) week 5 (39) week 4 (38.8) week 3 (16.3) week 2 (11.8) week 1 (11.6)

(A × B): Week 1 M (13.9) P (13.5) N (9.4) C (9.7)
Week 2 P (13.9) M (11.3) N (11.2) C (10.8)
Week 3 M (21.3) P (19.5) N (12.3) C (12.2)
Week 4 P (65.8) M (61.1) N (15) C (13.5)
Week 5 M (63.6) P (62.3) N (18.2) C (12.1)
Week 6 P (82.8) M (73.9) N (23.9) C (18.9)
Week 7 P (113.1) M (110.6) N (22.2) C (17)
Week 8 P (106.8) M (85.1) N (21.8) C (12.7)

*Based on Student–Newman–Keuls test: mean biomass (g per panel) of each treatment (M, P, N and C) are ranked from left to right in descending order; homogeneous groups ($P > 0.05$) are underlined and joined together.

(707.8 g), or trash fish, T (737.3 g), whereas for treatment N or without fish rearing (464.9 g), the fouling biomass was 40% less (Table 3). Despite the significantly higher biomass from sessile biofouling in net cages with fish rearing (M, T) as compared with unused net cages (N) in the first week, the difference was not significant for subsequent weeks.

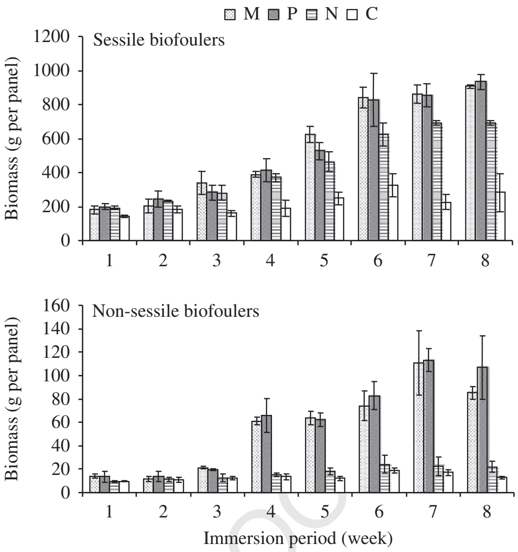

Figure 6 Weekly biomass (g per panel) of sessile biofoulers and non-sessile biofoulers (mean ± SD) on net panels by cage units given treatments M (with fish given commercially produced extruded pellet feed), P (with fish given home-made pellet feed), N (no fish and no feed) and C (outside net cage without given feed), during Experiment I. Each datum represents the mean value based on three replicate panels, each panel from three different cage units.

The biomass of both sessile and non-sessile biofouling organisms on the net panels was not significantly influenced by net cage position throughout the 8 weeks of study, i.e. with respect to the longitudinal (D–I–U) and cross-river (R–I–B) axes of the farm. Based on the results from the Latin Square ANOVA tests, the data were further analysed using a two-factor (feed and time) ANOVA with equal replication, after removing the 'position' factor. The ANOVA test provided more statistical power and was able to detect significantly higher fouling biomass in feed treatments M and T as compared with N. These were observed in 4 and 6 weeks out of 8 weeks for sessile and non-sessile biofouling, respectively, particularly after 4 weeks of immersion (see Table 3).

Discussion

Effects of fish rearing

Results from the present study suggest that fish rearing in floating net cages promotes biofouling by providing favourable conditions for the colonization and growth of biofouling organisms. The two main factors involved are organic matter input and a reduced-flow environment.

Table 3 Weekly development in mean biomass (g per panel, mean ± SD) of sessile and non-sessile biofoulers in Experiment II

	Weeks						
	1	2	3	4	5	6	7	8
Sessile								
M	219.0 ± 18.8 *[a]	284.1 ± 79.9	250.2 ± 19.5	305.8 ± 31.4 †[a]	443.9 ± 115.9	707.8 ± 100.6 †[a]	791.1 ± 21.2	817.7 ± 100.1 †[a]
T	222.3 ± 19.1*[a]	287.6 ± 119.6	218.8 ± 37.7	294.5 ± 38.7 †[a]	519.6 ± 48.8	737.3 ± 121.3 †[a]	713.7 ± 252.0	777.2 ± 122.2 †[a]
N	176.4 ± 7.0 *[b]	313.3 ± 59.2	314.4 ± 124.2	214.7 ± 34.7 †[b]	357.8 ± 39.5	464.9 ± 30.2 †[b]	498.3 ± 65.5	441.8 ± 135.4 †[b]
D	193.0 ± 13.5	242.2 ± 32.7	239.2 ± 60.3	261.4 ± 35.3	427.8 ± 126.2	615.6 ± 165.2	559.3 ± 205.4	618.2 ± 323.5
I	205.4 ± 32.2	283.1 ± 87.1	225.2 ± 37.7	262.9 ± 82.6	430.2 ± 57.8	637.9 ± 165.9	774.8 ± 201.1	707.3 ± 178.0
U	219.4 ± 31.6	359.8 ± 74.9	318.9 ± 107.9	290.7 ± 46.8	463.3 ± 128.8	657.1 ± 191.9	668.9 ± 140.8	711.0 ± 162.4
R	215.5 ± 33.2	290.6 ± 79.8	256.7 ± 45.9	259.4 ± 34.0	472.1 ± 79.4	673.5 ± 189.5	717.6 ± 267.4	642.3 ± 311.9
I	200.1 ± 17.0	249.8 ± 29.0	98.6 ± 132.1	287.6 ± 49.4	446.7 ± 126.2	543.8 ± 91.9	590.1 ± 191.8	635.8 ± 109.0
B	202.2 ± 33.7	344.6 ± 104.8	228.0 ± 31.8	268.1 ± 82.8	402.5 ± 105.3	693.3 ± 169.6	695.4 ± 122.7	758.5 ± 217.9
Non-sessile								
M	9.9 ± 1.2	20.0 ± 5.5 †[a]	55.2 ± 1.1	61.2 ± 11.2 †[a]	96.6 ± 7.0 *[a]	78.5 ± 11.0 *[a]	74.0 ± 10.5 *[a]	114.5 ± 40.8 †[a]
T	8.9 ± 0.5	13.9 ± 1.7 †[a]	42.8 ± 32.4	54.1 ± 22.3 †[a]	87.9 ± 8.2 *[a]	81.0 ± 11.8 *[a]	88.9 ± 14.8 *[a]	110.2 ± 25.3 †[a]
N	8.5 ± 0.2	9.5 ± 1.1 †[b]	10.8 ± 4.0	9.6 ± 0.5 †[b]	21.2 ± 12.9*[b]	26.7 ± 17.4 *[b]	25.9 ± 8.1 *[b]	20.5 ± 8.2 †[b]
D	9.1 ± 0.9	16.3 ± 5.8	24.4 ± 25.5	32.7 ± 20.5	73.9 ± 34.7	69.7 ± 20.1	64.2 ± 27.1	97.4 ± 70.5
I	9.8 ± 1.1	15.5 ± 7.8	36.4 ± 26.0	41.2 ± 30.3	63.9 ± 43.3	59.5 ± 36.6	65.4 ± 37.6	92.4 ± 54.3
U	8.5 ± 0.1	11.7 ± 2.2	48.0 ± 29.9	51.0 ± 37.1	67.9 ± 47.1	56.9 ± 38.7	59.2 ± 38.1	55.4 ± 36.2
R	8.8 ± 0.5	12.7 ± 1.7	37.6 ± 23.8	38.8 ± 27.4	71.7 ± 30.9	75.7 ± 25.3	67.7 ± 33.8	72.2 ± 54.8
I	9.4 ± 1.4	16.3 ± 7.4	26.6 ± 25.7	39.8 ± 30.8	62.1 ± 42.4	54.2 ± 35.9	52.8 ± 30.5	85.3 ± 61.7
B	9.1 ± 0.9	14.4 ± 7.1	44.7 ± 34.1	46.3 ± 34.9	71.9 ± 50.2	56.3 ± 32.0	68.3 ± 36.4	87.8 ± 61.5

Stocked fish fed commercially produced extruded pellet feed (M); stocked fish fed trash fish feed (T); and net cage without fish and feed (N). Net cage position along longitudinal axis from downstream (D), mid-position (I) and upstream (U); and cross-river axis from midstream (R), mid-position (I) and river bank (B). See Fig. 2b for further explanation.

[a, b] indicates homogeneous group.

*Significant difference among treatment feed (M, T, N) ($P < 0.05$), based on Latin Square Design ANOVA test.

†Significant difference among treatment feed (M, T, N) ($P < 0.05$), based on two-way ANOVA test after removing the 'position' factor.

Fish feed wastage and nutrient loading have been reported to be several times higher if minced trash fish is used instead of pellet feed (Wu 1995). Such an enrichment is expected to attract higher biofouling on net panels. Contrary to this expectation, the biomass of especially sessile biofoulers in net cages given a commercial pellet feed (M) was higher, but not significantly, than those given trash fish feed (T) (Experiment II), suggesting that biofouling is not significantly influenced by the type of fish feed input. This contention is also supported by results obtained in Experiment I where there was no significant difference in biofouling rate between the pellet feeds of different quality (M and P). Thus, even the use of high-quality feed (M) in fish rearing did not help to alleviate biofouling on the nets.

The results also indicate that the input of organic matter including feed and faeces from fish rearing is likely to increase sessile biofouling because net panels in cages without fish rearing (N) had lower rates (25% lower) of sessile biofouling as compared with those in cages with fish rearing (M and P) after 4 weeks of immersion (see Fig. 6). However, the lowest sessile biofouling rate (65% lower) occurred outside the cages (C), which must therefore be due to also the effect of water flow velocity.

Non-sessile or mobile organisms on the nettings, however, responded very differently. In feed-receiving net-cages (both M and P), non-sessile fouling rates increased by up to 80% higher than in net cages without fish rearing (N) or outside the net cages (C) after 3 weeks of immersion (Experiment I). Because the abundance of non-sessile organisms was about equal in N and C treatments (see Fig. 6), current velocity (higher in C than N) is not necessarily the main factor controlling non-sessile fouling. Instead, the non-sessile organisms appear to be strongly attracted to food availability sustained by fish rearing although an environment of reduced water flow appears conducive. The type of food essential for non-sessile organisms was, however, not assessed, e.g. whether they relied on feed wastage or faeces of cultivated fish. Both sources are likely consumed because coprophagy has been reported in zooplankton and is common among copepod species (Gonzalez & Smetacek 1994), while scavenging is common in several amphipod species (Britton & Morton 1994).

Effects of water flow

The water flow attenuation through an unfouled net cage of 16 mm mesh size was approximately 20% which is comparable with a 25% flow reduction through clean nets of 12–18 mm mesh sizes reported by Black (1998). With progressive biofouling, as high as 90% flow velocity diminution was obtained in this study after 3 weeks of biofouling. As a result of impedance, the water flow on encountering the floating net cage farm was significantly deflected to below the net cages, thereby increasing the flow velocity here (see Fig. 4). This mode of deflection explains why all net cages further into the leeside experienced very similar but weak flow to the extent that even net fouling did not significantly reduce the flow rate any further. It also explains why the position of experimental cages (Experiment II) did not affect fouling due to the rather slow but homogeneous surface flow rates ($< 10\ cm\ s^{-1}$) across the farm (see Fig. 4).

According to Black (1998), water movement is essential in caged fish culture for the removal of waste products. The impeded water flow through net cages will not easily remove organic inputs such as uneaten food particulates and fish faeces from the farm, further enhancing the growth of biofouling organisms. This probably happened in Experiment I because net panels in treatments P and M had a much higher biofouling than C. The relative effect of water velocity *vis-à-vis* organic input and their interaction on sessile biofouling rate are, however, difficult to assess because water flow is dynamic and organic input is subject to variable consumption, production and advection. Nevertheless, data suggest that water flow rate may be more critical than organic input because irrespective of the amount of feed given, a swift current will make the food less available to sessile biofoulers and more difficult for their larvae to colonize. The study suggests that if the mean high flow velocity through the net cages is weak ($< 10\ cm\ s^{-1}$), biofouling by sessile organisms with or without organic input will be high, but the mobile organisms will be more attracted to organic inputs from fish rearing. However, with stronger high flow ($> 25\ cm\ s^{-1}$) as occurring outside the net cages (C), sessile biofouling biomass could be significantly reduced by almost 65%. Results from Experiment I indicate that a mean high flow velocity of $25\ cm\ s^{-1}$ is probably near to the critical threshold below which biofouling rapidly develops. For instance, a 20% flow reduction (i.e. flow velocity of ca. $20\ cm\ s^{-1}$) in the clean nets of frontline net cages (net position 3, see Fig. 3) had initiated rapid biofouling, which further reduced the flow velocity by 75–80% ($< 10\ cm\ s^{-1}$) after 2 weeks of immersion. This drastic velocity reduction after 2 weeks of immersion apparently sets the pace for rapid sessile

biofouling in the subsequent weeks (see Fig. 6). On the other hand, outside the fish cage where the mean high flow velocity was $> 25\ \mathrm{cm\ s^{-1}}$, the net biofouling rate remained low and constant over weeks. The cover of *Balanus amphitrite* as well *Polysiphonia* sp. was significantly higher inside the net cage ($< 10\ \mathrm{cm\ s^{-1}}$) than outside it ($> 20\ \mathrm{cm\ s^{-1}}$) (see Madin *et al.* 2009). This finding is consistent with that reported by others. Qian, Rittschof & Sreedhar (2000) reported that larval settlement of barnacles was at its highest if flow rates were between 2.1 and $10.6\ \mathrm{cm\ s^{-1}}$ and larvae did not settle when the flow rate exceeded $21\ \mathrm{cm\ s^{-1}}$. Spore development and thallus growth of the alga *Gracilaria* are reduced by strong water flow of $> 13.7\ \mathrm{cm\ s^{-1}}$ (Ryder, Nelson, McKeona, Glenn, Fitzsimmons & Napolean 2004).

The water flow rate is known to have a significant impact on the form, behaviour, feeding, growth rates and final settlement of larvae and spores of aquatic organisms including all common biofouling species (e.g. Eckman & Duggins 1993; Okamura & Partridge 1999; Qian *et al.* 2000; Marchinko & Palmer 2003). In the present study, the higher biomass of sessile biofouling organisms on net panels placed inside the net cages as compared with those placed outside it suggests that the slower flow rate inside the net cages is more conducive to larval settlement of colonial biofouling organisms, while stronger flow rates as occurring outside the net cages could have reduced retention efficiency.

The reduced water flow is likely to enhance feeding and thus the growth rate of settled macrofoulers as they are in contact with food in the water column for a longer period. However, in strong water flow condition, the observed sessile biofoulers have to withstand drag forces and shear stress, which could reduce their growth rates and biomass. In addition, food depletion will occur and the macrofoulers have to compete for the fast-moving food. According to Okamura (1992), common biofouling organisms including bryozoans grow more slowly in strong water flow environments such as in wave-exposed habitats due to low rates of food supply and feeding efficiency.

The first 1–2 weeks appear critical in terms of spore or larval settlement and their subsequent colonization. Whether inside or outside the fish cages, the hydroid *Plumularia* sp. was the first visible macrofouler after 1 week of immersion, followed by a small sea anemone and the macroalgae, *Enteromorpha* and *Polysiphonia* after 2 weeks of immersion (Madin *et al.* 2009). Inside the cages, where water flow was slower, the algae grew very rapidly along with new visible colonization by barnacles and mussels in the subsequent weeks, while outside the cages where water flow was stronger, these species did not proliferate except for the small filamentous (*Lynbya* sp.) and stolonate (*Plumularia*) forms.

We estimate a mean total biofouling rate of $92\ \mathrm{g\ m^{-2}\ week^{-1}}$ outside the net cage, but reduced water flow inside the net cage could increase the total biofouling rate to $223\ \mathrm{g\ m^{-2}\ week^{-1}}$ (142%). Fish rearing using commercial pellet feed in a flow-reduced environment further increased the total biofouling rate to $310\ \mathrm{g\ m^{-2}\ week^{-1}}$ (237%) (Table 4). Our study concludes that the existing floating cage culture system of linearly arranged cage units severely hampers water flow through the fish farm, thereby promoting net biofouling and in consequence, poor oxygenation and water quality. The present cage culture design and operation also

Table 4 Summarized data of highest (eighth week) mean biomass of sessile and non-sessile biofouling organisms among treatment feeds in Experiment I (C, N, M and P) and Experiment II (N, T and M)

	Biofouling rates ($\mathrm{g\ m^{-2}}$)			Mean rate
	Sessile	Non-sessile	Total	($\mathrm{g\ m^{-2}\ week^{-1}}$)
Experiment I				
No fish, feed and enclosing cage netting (C)	704	31.7	735.7	92
Net cage without fish and feed (N)	1728.5	54.5	1783	223
Stocked fish fed commercially produced extruded pellet feed (M)	2265.2	212.7	2477.9	310
Stocked fish fed home-made pellet feed (P)	2331.7	267	2598.7	325
Experiment II				
Net cage without fish and feed (N)	11104.5	51.2	1155.7	145
Stocked fish fed trash fish feed (T)	1943	275.5	2217.5	277
Stocked fish fed commercially produced extruded pellet feed (M)	2044.2	286.2	2327.5	291

Mean biofouling rate based on total weight (g) on 1 m2 of net panel per week.

physically impact the river depth by increasing sedimentation under the farm area due to rapid ebb flow attenuation (see Fig. 4). Evidence of shallowing is seen in the Sangga Besar river where cage culture began in the late 1980s and is now no longer accessible to large boats during low tide. These problems are further exacerbated by the concentration of many fish farms of similar design in tropical estuaries. Hydrodynamics and cage array design are very important considerations in site selection and operation of floating cage farms. The study recommends that square net cages in tidal estuaries, where the dominant flows are bi-directional, should not be serially arranged in a grid form. Instead, they should be arranged in order to increase the surface area of contact with the incoming current, as well as increasing space between cages in order to improve flow through. One example that could be further researched on is the tilted checker board design with two opposite corners aligned along the long axis of the river. The 'white squares' would create space to increase water flow through the 'black squares' (cage units).

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APPENDIX 8

Summary of 3-Way ANOVA and *Post Hoc* Test Results on the Effects of Fish Feed (M = Commercially-Produced Pellet, P = Home-Made Pellet, N = Without Fish & Feed, C = Outside Cages), Immersion Time (Wk 0, 2, 4, 6, 8) and Position (A, 3, 2, 1, B) on the Water Flow Velocity (Experiment I)

Appendix 8

Summary of 3-Way ANOVA and Post Hoc Test Results on the Effects of Fish Feed (M = Commercially-Produced Pellet, P = Home-Made Pellet, N = Without Fish & Feed, C = Outside Cages), Immersion Time (Wk 0, 2, 4, 6, 8) and Position (A, 3, 2, 1, B) on the Water Flow Velocity (Experiment I)

ANOVA results

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Week	4	1122.68	486	3.03	371.13	0.0000
Treatment	3	13454.66	486	3.03	4447.81	0.0000
Position	4	9249.73	486	3.03	3057.76	0.0000
Week x Treatments	12	426.27	486	3.03	140.91	0.0000
Week x Position	16	291.19	486	3.03	96.26	0.0000
Treatments x Position	12	788.54	486	3.03	260.67	0.0000
Week x Treatments x Position	48	35.76	486	3.03	11.82	0.0000

Post hoc test results (Student – Newman – Keuls test)

Week	Treatment	Position		{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}	{17}	{18}	{19}	{20}
				26.25	21.25	6.50	5.25	6.25	27.25	21.75	6.50	5.25	5.75	26.50	27.00	27.00	25.29	25.20	27.25	21.40	6.40	4.00	5.33
0	M	A	{1}		0.0002	0.0000	0.0000	0.0000	0.9720	0.0007	0.0000	0.0000	0.0000	0.9722	0.9612	0.9844	0.3831	0.6088	0.9857	0.0002	0.0000	0.0000	0.0000
0	M	3	{2}	0.0002		0.0000	0.0000	0.0000	0.0000	0.8934	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0049	0.0048	0.0000	0.8921	0.0000	0.0000	0.0000
0	M	2	{3}	0.0000	0.0000		0.9995	0.9994	0.0000	0.0000	1.0000	0.9992	0.9996	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9279	0.8802	0.9988
0	M	1	{4}	0.0000	0.0000	0.9995		0.9996	0.0000	0.0000	0.9997	1.0000	0.9998	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9997	0.9890	0.9998
0	M	B	{5}	0.0000	0.0000	0.9994	0.9996		0.0000	0.0000	0.9999	0.9991	0.9976	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9991	0.9272	0.9981
0	P	A	{6}	0.9720	0.0000	0.0000	0.0000	0.0000		0.0001	0.0000	0.0000	0.0000	0.9612	0.9722	0.8211	0.6361	0.6456	1.0000	0.0000	0.0000	0.0001	0.0000
0	P	3	{7}	0.0007	0.8934	0.0000	0.0000	0.0000	0.0001		0.0000	0.0000	0.0000	0.0005	0.0001	0.0001	0.0121	0.0098	0.0001	0.7516	0.0000	0.0000	0.0000
0	P	2	{8}	0.0000	0.0000	1.0000	0.9997	0.9999	0.0000	0.0000		0.9995	0.9999	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9955	0.8917	0.9993
0	P	1	{9}	0.0000	0.0000	0.9992	1.0000	0.9991	0.0000	0.0000	0.9995		0.9994	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9994	0.9934	0.9969
0	P	B	{10}	0.0000	0.0000	0.9996	0.9998	0.9976	0.0000	0.0000	0.9999	0.9994		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9997	0.9837	0.9957

0	N	A	{11}	0.9722	0.0001	0.0000	0.0000	0.0000	0.9612	0.0005	0.0000	0.0000	0.0000		0.8934	0.9692	0.6906	0.7653	0.9844	0.0002	0.0000	0.0000	0.0000
0	N	3	{12}	0.9612	0.0000	0.0000	0.0000	0.0000	0.9722	0.0001	0.0000	0.0000	0.0000	0.8934		1.0000	0.6314	0.6641	0.9959	0.0000	0.0000	0.0000	0.0000
0	N	2	{13}	0.9844	0.0000	0.0000	0.0000	0.0000	0.8211	0.0001	0.0000	0.0000	0.0000	0.9692	1.0000		0.7140	0.7334	0.9722	0.0000	0.0000	0.0001	0.0000
0	N	1	{14}	0.3831	0.0049	0.0000	0.0000	0.0000	0.6361	0.0121	0.0000	0.0000	0.0000	0.6906	0.6314	0.7140		0.9382	0.6979	0.0059	0.0000	0.0000	0.0000
0	N	B	{15}	0.6088	0.0048	0.0000	0.0000	0.0000	0.6456	0.0098	0.0000	0.0000	0.0000	0.7653	0.6641	0.7334	0.9382		0.7002	0.0053	0.0000	0.0000	0.0000
0	C	A	{16}	0.9857	0.0000	0.0000	0.0000	0.0000	1.0000	0.0001	0.0000	0.0000	0.0000	0.9844	0.9959	0.9722	0.6979	0.7002		0.0000	0.0000	0.0001	0.0000
0	C	3	{17}	0.0002	0.8921	0.0000	0.0000	0.0000	0.0000	0.7516	0.0000	0.0000	0.0000	0.0002	0.0000	0.0000	0.0059	0.0053	0.0000		0.0000	0.0000	0.0000
0	C	2	{18}	0.0000	0.0000	0.9279	0.9997	0.9991	0.0000	0.0000	0.9955	0.9994	0.9997	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.9087	0.9991
0	C	1	{19}	0.0000	0.0000	0.8802	0.9890	0.9272	0.0001	0.0000	0.8917	0.9934	0.9837	0.0000	0.0000	0.0001	0.0000	0.0000	0.0001	0.0000	0.9087		0.9955
0	C	B	{20}	0.0000	0.0000	0.9988	0.9998	0.9981	0.0000	0.0000	0.9993	0.9969	0.9957	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9991	0.9955	
2	M	A	{21}	0.9692	0.0000	0.0000	0.0000	0.0000	0.9692	0.0002	0.0000	0.0000	0.0000	0.8211	0.8211	0.9722	0.6760	0.7260	0.9914	0.0001	0.0000	0.0000	0.0000
2	M	3	{22}	0.0000	0.0000	0.9722	0.9980	0.9994	0.0000	0.0000	0.8211	0.9971	0.9991	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9890	0.7750	0.9966
2	M	2	{23}	0.0000	0.0000	0.9455	0.9160	0.9817	0.0000	0.0000	0.8953	0.9032	0.9626	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9553	0.2922	0.9046
2	M	1	{24}	0.0000	0.0000	0.9611	0.9054	0.9716	0.0000	0.0000	0.9682	0.9612	0.9890	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9709	0.9998	0.9891
2	M	B	{25}	0.0000	0.0000	0.9990	1.0000	0.9914	0.0000	0.0000	0.9996	0.9998	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9990	0.9877	0.9990
2	P	A	{26}	0.8146	0.0000	0.0000	0.0000	0.0000	0.7761	0.0000	0.0000	0.0000	0.0000	0.8248	0.8953	0.8025	0.2933	0.2855	0.4976	0.0000	0.0000	0.0001	0.0000
2	P	3	{27}	0.0000	0.0000	0.9976	1.0000	0.9692	0.0000	0.0000	0.9990	1.0000	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9972	0.9907	0.9998
2	P	2	{28}	0.0000	0.0000	0.9986	1.0000	0.9982	0.0000	0.0000	0.9992	1.0000	0.9976	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9990	0.9960	0.9399
2	P	1	{29}	0.0000	0.0000	0.7898	0.9865	0.8676	0.0001	0.0000	0.8036	0.9907	0.9682	0.0001	0.0001	0.0001	0.0000	0.0000	0.0001	0.0000	0.8329	0.9959	0.9922
2	P	B	{30}	0.0000	0.0000	0.9272	0.9720	0.9525	0.0000	0.0000	0.9369	0.9857	0.9865	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9452	0.9994	0.9934
2	N	A	{31}	1.0000	0.0002	0.0000	0.0000	0.0000	0.9455	0.0009	0.0000	0.0000	0.0000	0.8211	0.9054	0.9612	0.6579	0.7779	0.9720	0.0003	0.0000	0.0000	0.0000
2	N	3	{32}	0.7752	0.0000	0.0000	0.0000	0.0000	0.8953	0.0000	0.0000	0.0000	0.0000	0.8146	0.9189	0.8688	0.2356	0.2219	0.8025	0.0000	0.0000	0.0001	0.0000
2	N	2	{33}	0.8136	0.0000	0.0000	0.0000	0.0000	0.9455	0.0000	0.0000	0.0000	0.0000	0.8569	0.9503	0.9189	0.2628	0.2459	0.8953	0.0000	0.0000	0.0001	0.0000
2	N	1	{34}	0.0935	0.1751	0.0000	0.0000	0.0000	0.0289	0.1135	0.0000	0.0000	0.0000	0.0949	0.0412	0.0497	0.3700	0.2733	0.0338	0.1389	0.0000	0.0000	0.0000
2	N	B	{35}	0.6706	0.0063	0.0000	0.0000	0.0000	0.5740	0.0092	0.0000	0.0000	0.0000	0.7530	0.6140	0.6764	0.9639	0.8565	0.6242	0.0062	0.0000	0.0000	0.0000
2	C	A	{36}	0.8569	0.0000	0.0000	0.0000	0.0000	0.9054	0.0000	0.0000	0.0000	0.0000	0.8767	0.9455	0.8953	0.3316	0.3195	0.7761	0.0000	0.0000	0.0001	0.0000
2	C	3	{37}	0.0000	0.0000	0.9957	1.0000	0.9128	0.0000	0.0000	0.9984	1.0000	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9944	0.9902	0.9999
2	C	2	{38}	0.0000	0.0000	0.9997	0.9996	0.9990	0.0000	0.0000	0.9998	0.9978	0.9899	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9997	0.9878	0.9685
2	C	1	{39}	0.0000	0.0000	0.9106	0.9774	0.9426	0.0000	0.0000	0.9213	0.9877	0.9846	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9322	0.9988	0.9936
2	C	B	{40}	0.0000	0.0000	0.9993	0.9399	0.9995	0.0000	0.0000	0.9996	0.9969	0.9999	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9996	0.9887	0.9999
4	M	A	{41}	0.0094	0.0000	0.0000	0.0001	0.0000	0.0939	0.0000	0.0000	0.0001	0.0000	0.0177	0.0630	0.0556	0.0002	0.0002	0.0814	0.0000	0.0000	0.0000	0.0000
4	M	3	{42}	0.0000	0.0000	0.9921	0.9977	0.9996	0.0000	0.0000	0.9569	0.9967	0.9992	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9959	0.7503	0.9963
4	M	2	{43}	0.0001	0.0000	0.2874	0.8429	0.4019	0.0000	0.0000	0.2978	0.8618	0.6820	0.0001	0.0001	0.0000	0.0001	0.0001	0.0000	0.0000	0.3392	0.9892	0.8572

4	M	1	{44}	0.0001	0.0000	0.1115	0.6424	0.1810	0.0000	0.0001	0.1160	0.6648	0.4158	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.0001	0.1401	0.9714	0.6476
4	M	B	{45}	0.0001	0.0000	0.3150	0.8524	0.4313	0.0000	0.0000	0.3264	0.8716	0.7059	0.0001	0.0001	0.0001	0.0001	0.0001	0.0000	0.0000	0.3686	0.9857	0.8694
4	P	A	{46}	0.0166	0.0000	0.0000	0.0001	0.0000	0.1310	0.0000	0.0000	0.0001	0.0000	0.0294	0.0935	0.0820	0.0005	0.0004	0.1126	0.0000	0.0000	0.0000	0.0000
4	P	3	{47}	0.0000	0.0000	0.9409	0.9622	0.9604	0.0000	0.0000	0.9497	0.9820	0.9878	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9557	0.9997	0.9928
4	P	2	{48}	0.0001	0.0000	0.2205	0.7910	0.3231	0.0000	0.0000	0.2288	0.8117	0.6015	0.0001	0.0000	0.0000	0.0001	0.0001	0.0000	0.0000	0.2655	0.9862	0.8034
4	P	1	{49}	0.0000	0.0000	0.0011	0.0576	0.0027	0.0000	0.0000	0.0011	0.0605	0.0152	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0016	0.5570	0.0515
4	P	B	{50}	0.0000	0.0000	0.0339	0.4466	0.0655	0.0000	0.0000	0.0352	0.4616	0.2113	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0456	0.9682	0.4288
4	N	A	{51}	0.0026	0.0000	0.0000	0.0001	0.0000	0.0389	0.0000	0.0000	0.0001	0.0000	0.0054	0.0236	0.0208	0.0001	0.0001	0.0338	0.0000	0.0000	0.0000	0.0001
4	N	3	{52}	0.1085	0.0000	0.0000	0.0000	0.0000	0.3603	0.0000	0.0000	0.0000	0.0000	0.1539	0.3185	0.2772	0.0066	0.0054	0.3032	0.0000	0.0000	0.0000	0.0000
4	N	2	{53}	0.0001	0.0000	0.0000	0.0001	0.0000	0.0014	0.0000	0.0000	0.0001	0.0000	0.0001	0.0007	0.0006	0.0000	0.0000	0.0012	0.0000	0.0000	0.0000	0.0001
4	N	1	{54}	0.1209	0.0000	0.0000	0.0000	0.0000	0.4150	0.0000	0.0000	0.0000	0.0000	0.1735	0.3587	0.3185	0.0074	0.0061	0.3603	0.0000	0.0000	0.0000	0.0000
4	N	B	{55}	0.0203	0.0000	0.0000	0.0001	0.0000	0.1401	0.0000	0.0000	0.0000	0.0000	0.0347	0.1041	0.0904	0.0006	0.0005	0.1186	0.0000	0.0000	0.0000	0.0000
4	C	A	{56}	0.1439	0.0000	0.0000	0.0000	0.0000	0.3924	0.0000	0.0000	0.0000	0.0000	0.1937	0.3661	0.3154	0.0103	0.0087	0.3223	0.0000	0.0000	0.0001	0.0000
4	C	3	{57}	0.0000	0.0000	0.9697	0.9757	0.9949	0.0000	0.0000	0.9214	0.9698	0.9901	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9791	0.4835	0.9691
4	C	2	{58}	0.0000	0.0000	0.7902	0.9779	0.8629	0.0001	0.0000	0.8050	0.9852	0.9626	0.0000	0.0001	0.0001	0.0000	0.0000	0.0001	0.0000	0.8318	0.8565	0.9886
4	C	1	{59}	0.0000	0.0001	0.0524	0.5205	0.0956	0.0000	0.0001	0.0544	0.5380	0.2751	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0689	0.9740	0.5085
4	C	B	{60}	0.0001	0.0000	0.1662	0.7323	0.2549	0.0000	0.0001	0.1727	0.7541	0.5205	0.0001	0.0000	0.0000	0.0001	0.0001	0.0000	0.0000	0.2040	0.9820	0.7416
6	M	A	{61}	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
6	M	3	{62}	0.0000	0.0000	0.8676	0.9817	0.9160	0.0001	0.0000	0.8802	0.9890	0.9785	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.8975	1.0000	0.9928
6	M	2	{63}	0.0000	0.0001	0.0327	0.4313	0.0630	0.0000	0.0000	0.0339	0.4466	0.2037	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0439	0.9611	0.4152
6	M	1	{64}	0.0000	0.0000	0.0056	0.1659	0.0125	0.0000	0.0000	0.0058	0.1734	0.0558	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0080	0.8035	0.1534
6	M	B	{65}	0.0000	0.0000	0.9988	0.9995	0.9399	0.0000	0.0000	0.9999	0.9990	0.9985	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9980	0.9106	0.9982
6	P	A	{66}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
6	P	3	{67}	0.0000	0.0000	0.9945	0.9995	0.9913	0.0000	0.0000	0.9996	0.9991	0.9991	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9920	0.9017	0.9985
6	P	2	{68}	0.0001	0.0000	0.5242	0.9382	0.6444	0.0001	0.0000	0.5395	0.9505	0.8633	0.0001	0.0001	0.0001	0.0000	0.0000	0.0001	0.0000	0.5841	0.9909	0.9525
6	P	1	{69}	0.0000	0.0001	0.0601	0.5437	0.1075	0.0000	0.0001	0.0624	0.5620	0.2978	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0784	0.9750	0.5341
6	P	B	{70}	0.0000	0.0000	0.9996	0.9998	0.9985	0.0000	0.0000	0.9998	0.9990	0.9399	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9997	0.9867	0.9905
6	N	A	{71}	0.0000	0.0000	0.0001	0.0000	0.0001	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
6	N	3	{72}	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6	N	2	{73}	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6	N	1	{74}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
6	N	B	{75}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
6	C	A	{76}	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001

6	C	3	{77}	0.0000	0.0000	0.9944	1.0000	0.7516	0.0000	0.0000	0.9982	1.0000	0.9999	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9914	0.9855	0.9999
6	C	2	{78}	0.0000	0.0001	0.0911	0.6150	0.1532	0.0000	0.0001	0.0947	0.6357	0.3759	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.0001	0.1160	0.9763	0.6138
6	C	1	{79}	0.0000	0.0001	0.0315	0.4158	0.0606	0.0000	0.0000	0.0327	0.4313	0.1961	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0423	0.9525	0.4014
6	C	B	{80}	0.0000	0.0000	0.9979	1.0000	0.9960	0.0000	0.0000	0.9988	0.9998	0.9818	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9984	0.9972	1.0000
8	M	A	{81}	0.0000	0.0000	0.0001	0.0000	0.0001	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
8	M	3	{82}	0.0000	0.0000	0.0326	0.0032	0.0304	0.0000	0.0000	0.0263	0.0029	0.0109	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0296	0.0001	0.0034
8	M	2	{83}	0.0000	0.0001	0.0906	0.6245	0.1533	0.0000	0.0001	0.0941	0.6443	0.3791	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0001	0.1156	0.9814	0.6209
8	M	1	{84}	0.0000	0.0000	0.8537	0.9697	0.9032	0.0000	0.0000	0.8676	0.9817	0.9716	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.8849	1.0000	0.9886
8	M	B	{85}	0.0000	0.0000	0.9547	0.9464	0.9686	0.0000	0.0000	0.9623	0.9765	0.9894	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9663	0.9997	0.9922
8	P	A	{86}	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
8	P	3	{87}	0.0000	0.0000	0.0861	0.0154	0.0913	0.0000	0.0000	0.0684	0.0142	0.0416	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0823	0.0002	0.0160
8	P	2	{88}	0.0000	0.0001	0.0810	0.6070	0.1395	0.0000	0.0001	0.0841	0.6261	0.3573	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.1041	0.9819	0.6005
8	P	1	{89}	0.0001	0.0000	0.4764	0.9292	0.6005	0.0001	0.0000	0.4908	0.9419	0.8385	0.0001	0.0001	0.0001	0.0001	0.0000	0.0000	0.0000	0.5368	0.9938	0.9426
8	P	B	{90}	0.0000	0.0000	0.7750	0.9803	0.8537	0.0001	0.0000	0.7898	0.9865	0.9611	0.0000	0.0001	0.0001	0.0000	0.0000	0.0001	0.0000	0.8191	0.9722	0.9890
8	N	A	{91}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
8	N	3	{92}	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
8	N	2	{93}	0.0000	0.0000	0.0001	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
8	N	1	{94}	0.0000	0.0000	0.0001	0.0000	0.0001	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
8	N	B	{95}	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
8	C	A	{96}	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
8	C	3	{97}	0.0000	0.0000	0.1812	0.0576	0.2145	0.0000	0.0000	0.1420	0.0535	0.1232	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1817	0.0012	0.0581
8	C	2	{98}	0.0001	0.0001	0.0875	0.5934	0.1470	0.0000	0.0001	0.0911	0.6150	0.3619	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.0001	0.1115	0.9670	0.5947
8	C	1	{99}	0.0000	0.0000	0.6342	0.9626	0.7425	0.0001	0.0000	0.6501	0.9716	0.9160	0.0001	0.0001	0.0001	0.0000	0.0000	0.0001	0.0000	0.6906	0.9914	0.9740
8	C	B	{100}	0.0000	0.0000	0.9525	0.7761	0.9626	0.0000	0.0000	0.9611	0.9054	0.9817	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9635	1.0000	0.9750

(Cont.)

				{21}	{22}	{23}	{24}	{25}	{26}	{27}	{28}	{29}	{30}	{31}	{32}	{33}	{34}	{35}	{36}	{37}	{38}	{39}	{40}
Week	Treatments	Position		26.75	6.75	7.50	4.50	5.75	28.00	5.75	5.25	3.75	4.25	26.25	28.25	28.25	23.50	25.00	28.00	5.80	5.60	4.17	5.17
0	M	A	{1}	0.9692	0.0000	0.0000	0.0000	0.0000	0.8146	0.0000	0.0000	0.0000	0.0000	1.0000	0.7752	0.8136	0.0935	0.6706	0.8569	0.0000	0.0000	0.0000	0.0000
0	M	3	{2}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.0000	0.0000	0.1751	0.0063	0.0000	0.0000	0.0000	0.0000	0.0000
0	M	2	{3}	0.0000	0.9722	0.9455	0.9611	0.9990	0.0000	0.9976	0.9986	0.7898	0.9272	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9957	0.9997	0.9106	0.9993
0	M	1	{4}	0.0000	0.9980	0.9160	0.9054	1.0000	0.0000	1.0000	1.0000	0.9865	0.9720	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.9996	0.9774	0.9399
0	M	B	{5}	0.0000	0.9994	0.9817	0.9716	0.9914	0.0000	0.9692	0.9982	0.8676	0.9525	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9128	0.9990	0.9426	0.9995
0	P	A	{6}	0.9692	0.0000	0.0000	0.0000	0.0000	0.7761	0.0000	0.0000	0.0001	0.0000	0.9455	0.8953	0.9455	0.0289	0.5740	0.9054	0.0000	0.0000	0.0000	0.0000

0	P	3	{7}	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0009	0.0000	0.0000	0.1135	0.0092	0.0000	0.0000	0.0000	0.0000	0.0000
0	P	2	{8}	0.0000	0.8211	0.8953	0.9682	0.9996	0.0000	0.9990	0.9992	0.8036	0.9369	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9984	0.9998	0.9213	0.9996
0	P	1	{9}	0.0000	0.9971	0.9032	0.9612	0.9998	0.0000	1.0000	1.0000	0.9907	0.9857	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.9978	0.9877	0.9969
0	P	B	{10}	0.0000	0.9991	0.9626	0.9890	1.0000	0.0000	1.0000	0.9976	0.9682	0.9865	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.9899	0.9846	0.9999
0	N	A	{11}	0.8211	0.0000	0.0000	0.0000	0.0000	0.8248	0.0000	0.0000	0.0001	0.0000	0.8211	0.8146	0.8569	0.0949	0.7530	0.8767	0.0000	0.0000	0.0000	0.0000
0	N	3	{12}	0.8211	0.0000	0.0000	0.0000	0.0000	0.8953	0.0000	0.0000	0.0001	0.0000	0.9054	0.9189	0.9503	0.0412	0.6140	0.9455	0.0000	0.0000	0.0000	0.0000
0	N	2	{13}	0.9722	0.0000	0.0000	0.0000	0.0000	0.8025	0.0000	0.0000	0.0001	0.0000	0.9612	0.8688	0.9189	0.0497	0.6764	0.8953	0.0000	0.0000	0.0000	0.0000
0	N	1	{14}	0.6760	0.0000	0.0000	0.0000	0.0000	0.2933	0.0000	0.0000	0.0000	0.0000	0.6579	0.2356	0.2628	0.3700	0.9639	0.3316	0.0000	0.0000	0.0000	0.0000
0	N	B	{15}	0.7260	0.0000	0.0000	0.0000	0.0000	0.2855	0.0000	0.0000	0.0000	0.0000	0.7779	0.2219	0.2459	0.2733	0.8565	0.3195	0.0000	0.0000	0.0000	0.0000
0	C	A	{16}	0.9914	0.0000	0.0000	0.0000	0.0000	0.4976	0.0000	0.0000	0.0001	0.0000	0.9720	0.8025	0.8953	0.0338	0.6242	0.7761	0.0000	0.0000	0.0000	0.0000
0	C	3	{17}	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0003	0.0000	0.0000	0.1389	0.0062	0.0000	0.0000	0.0000	0.0000	0.0000
0	C	2	{18}	0.0000	0.9890	0.9553	0.9709	0.9990	0.0000	0.9972	0.9990	0.8329	0.9452	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9944	0.9997	0.9322	0.9996
0	C	1	{19}	0.0000	0.7750	0.2922	0.9998	0.9877	0.0001	0.9907	0.9960	0.9959	0.9994	0.0000	0.0001	0.0001	0.0000	0.0000	0.0001	0.9902	0.9878	0.9988	0.9887
0	C	B	{20}	0.0000	0.9966	0.9046	0.9891	0.9990	0.0000	0.9998	0.9399	0.9922	0.9934	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9999	0.9685	0.9936	0.9999
2	M	A	{21}		0.0000	0.0000	0.0000	0.0000	0.8688	0.0000	0.0000	0.0001	0.0000	0.8934	0.8767	0.9138	0.0650	0.6935	0.9189	0.0000	0.0000	0.0000	0.0000
2	M	3	{22}	0.0000		0.9054	0.9160	0.9982	0.0000	0.9964	0.9957	0.6501	0.8537	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9949	0.9990	0.8259	0.9973
2	M	2	{23}	0.0000	0.9054		0.5257	0.9509	0.0000	0.9356	0.8885	0.1885	0.4000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9303	0.9541	0.3587	0.8985
2	M	1	{24}	0.0000	0.9160	0.5257		0.9934	0.0000	0.9960	0.9844	0.9999	0.9959	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9965	0.9865	0.9982	0.8185
2	M	B	{25}	0.0000	0.9982	0.9509	0.9934		0.0000	1.0000	0.9994	0.9741	0.9907	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9989	0.9991	0.9890	1.0000
2	P	A	{26}	0.8688	0.0000	0.0000	0.0000	0.0000		0.0000	0.0000	0.0001	0.0000	0.7609	0.9722	0.9959	0.0032	0.2194	1.0000	0.0000	0.0000	0.0001	0.0000
2	P	3	{27}	0.0000	0.9964	0.9356	0.9960	1.0000	0.0000		0.9998	0.9788	0.9937	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9639	0.9999	0.9922	1.0000
2	P	2	{28}	0.0000	0.9957	0.8885	0.9844	0.9994	0.0000	0.9998		0.9937	0.9928	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9999	0.9890	0.9934	0.9998
2	P	1	{29}	0.0001	0.6501	0.1885	0.9999	0.9741	0.0001	0.9788	0.9937		0.9998	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9769	0.9778	0.9998	0.9878
2	P	B	{30}	0.0000	0.8537	0.4000	0.9959	0.9907	0.0000	0.9937	0.9928	0.9998		0.0000	0.0001	0.0001	0.0000	0.0000	0.0001	0.9938	0.9874	0.9399	0.9622
2	N	A	{31}	0.8934	0.0000	0.0000	0.0000	0.0000	0.7609	0.0000	0.0000	0.0000	0.0000		0.7298	0.7752	0.1278	0.7904	0.8146	0.0000	0.0000	0.0000	0.0000
2	N	3	{32}	0.8767	0.0000	0.0000	0.0000	0.0000	0.9722	0.0000	0.0000	0.0000	0.0001	0.7298		1.0000	0.0016	0.1610	0.8211	0.0000	0.0000	0.0001	0.0000
2	N	2	{33}	0.9138	0.0000	0.0000	0.0000	0.0000	0.9959	0.0000	0.0000	0.0000	0.0001	0.7752	1.0000		0.0019	0.1782	0.9722	0.0000	0.0000	0.0001	0.0000
2	N	1	{34}	0.0650	0.0000	0.0000	0.0000	0.0000	0.0032	0.0000	0.0000	0.0000	0.0000	0.1278	0.0016	0.0019		0.1749	0.0037	0.0000	0.0000	0.0000	0.0000
2	N	B	{35}	0.6935	0.0000	0.0000	0.0000	0.0000	0.2194	0.0000	0.0000	0.0000	0.0000	0.7904	0.1610	0.1782	0.1749		0.2452	0.0000	0.0000	0.0000	0.0000
2	C	A	{36}	0.9189	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	0.0000	0.0001	0.0001	0.8146	0.8211	0.9722	0.0037	0.2452		0.0000	0.0000	0.0001	0.0000
2	C	3	{37}	0.0000	0.9949	0.9303	0.9965	0.9989	0.0000	0.9639	0.9999	0.9769	0.9938	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		1.0000	0.9922	1.0000
2	C	2	{38}	0.0000	0.9990	0.9541	0.9865	0.9991	0.0000	0.9999	0.9890	0.9778	0.9874	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000		0.9865	0.9997
2	C	1	{39}	0.0000	0.8259	0.3587	0.9982	0.9890	0.0001	0.9922	0.9934	0.9998	0.9399	0.0000	0.0001	0.0001	0.0000	0.0000	0.0001	0.9922	0.9865		0.9720

2	C	B	{40}	0.0000	0.9973	0.8985	0.8185	1.0000	0.0000	1.0000	0.9998	0.9878	0.9622	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.9997	0.9720	
4	M	A	{41}	0.0346	0.0000	0.0000	0.0001	0.0000	0.3414	0.0000	0.0001	0.0000	0.0000	0.0085	0.3839	0.3244	0.0000	0.0001	0.2982	0.0000	0.0000	0.0000	0.0001
4	M	3	{42}	0.0000	0.9549	0.8082	0.9063	0.9984	0.0000	0.9970	0.9953	0.6188	0.8369	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9960	0.9990	0.8065	0.9968
4	M	2	{43}	0.0001	0.1751	0.0207	0.9904	0.7004	0.0000	0.7180	0.8785	0.9776	0.9938	0.0001	0.0000	0.0000	0.0000	0.0001	0.0000	0.7004	0.7443	0.9943	0.8620
4	M	1	{44}	0.0000	0.0582	0.0046	0.9525	0.4313	0.0000	0.4466	0.6859	0.9697	0.9716	0.0001	0.0000	0.0000	0.0001	0.0001	0.0000	0.4251	0.4903	0.9754	0.6770
4	M	B	{45}	0.0001	0.1961	0.0246	0.9907	0.7248	0.0000	0.7425	0.8885	0.9612	0.9934	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.7264	0.7640	0.9936	0.8694
4	P	A	{46}	0.0543	0.0000	0.0000	0.0001	0.0000	0.4078	0.0000	0.0000	0.0000	0.0001	0.0148	0.4337	0.3595	0.0000	0.0002	0.3537	0.0000	0.0000	0.0000	0.0001
4	P	3	{47}	0.0000	0.8778	0.4420	0.9876	0.9920	0.0000	0.9947	0.9915	0.9999	0.9399	0.0000	0.0001	0.0001	0.0000	0.0000	0.0000	0.9950	0.9878	0.9876	0.9436
4	P	2	{48}	0.0001	0.1277	0.0132	0.9838	0.6199	0.0000	0.6376	0.8305	0.9784	0.9901	0.0001	0.0000	0.0000	0.0001	0.0001	0.0000	0.6177	0.6713	0.9911	0.8153
4	P	1	{49}	0.0000	0.0004	0.0001	0.3293	0.0158	0.0000	0.0165	0.0635	0.6640	0.4539	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0144	0.0235	0.4962	0.0703
4	P	B	{50}	0.0000	0.0151	0.0008	0.8917	0.2189	0.0000	0.2265	0.4764	0.9837	0.9454	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2083	0.2752	0.9572	0.4924
4	N	A	{51}	0.0117	0.0000	0.0000	0.0001	0.0000	0.1937	0.0000	0.0001	0.0000	0.0000	0.0023	0.2380	0.2007	0.0000	0.0000	0.1681	0.0000	0.0000	0.0000	0.0001
4	N	3	{52}	0.2291	0.0000	0.0000	0.0001	0.0000	0.6595	0.0000	0.0000	0.0000	0.0001	0.0964	0.5749	0.4058	0.0000	0.0029	0.5576	0.0000	0.0000	0.0001	0.0000
4	N	2	{53}	0.0003	0.0000	0.0000	0.0001	0.0000	0.0156	0.0000	0.0001	0.0000	0.0000	0.0001	0.0243	0.0200	0.0000	0.0000	0.0133	0.0000	0.0000	0.0000	0.0001
4	N	1	{54}	0.2583	0.0000	0.0000	0.0001	0.0000	0.7406	0.0000	0.0000	0.0000	0.0001	0.1085	0.7028	0.5749	0.0000	0.0032	0.6595	0.0000	0.0000	0.0001	0.0001
4	N	B	{55}	0.0623	0.0000	0.0000	0.0001	0.0000	0.4080	0.0000	0.0000	0.0000	0.0001	0.0180	0.4119	0.3258	0.0000	0.0003	0.3464	0.0000	0.0000	0.0001	0.0001
4	C	A	{56}	0.2752	0.0000	0.0000	0.0001	0.0000	0.6555	0.0000	0.0000	0.0000	0.0001	0.1272	0.4952	0.2583	0.0000	0.0048	0.5267	0.0000	0.0000	0.0001	0.0000
4	C	3	{57}	0.0000	0.9128	0.7862	0.7198	0.9852	0.0000	0.9779	0.9626	0.3474	0.6015	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9744	0.9878	0.5579	0.9690
4	C	2	{58}	0.0000	0.6546	0.1956	0.9994	0.9698	0.0001	0.9757	0.9901	0.9989	0.9986	0.0000	0.0001	0.0001	0.0000	0.0000	0.0001	0.9741	0.9719	0.9974	0.9785
4	C	1	{59}	0.0000	0.0246	0.0015	0.9213	0.2851	0.0000	0.2951	0.5550	0.9846	0.9600	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2746	0.3462	0.9682	0.5644
4	C	B	{60}	0.0000	0.0919	0.0084	0.9740	0.5380	0.0000	0.5550	0.7742	0.9774	0.9846	0.0001	0.0000	0.0000	0.0001	0.0001	0.0000	0.5336	0.5947	0.9865	0.7616
6	M	A	{61}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
6	M	3	{62}	0.0000	0.7592	0.2807	0.9994	0.9837	0.0001	0.9877	0.9934	0.9994	0.9959	0.0000	0.0001	0.0001	0.0000	0.0000	0.0001	0.9872	0.9830	0.9876	0.9803
6	M	2	{63}	0.0000	0.0146	0.0008	0.8802	0.2113	0.0000	0.2189	0.4616	0.9785	0.9369	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2013	0.2655	0.9497	0.4760
6	M	1	{64}	0.0000	0.0022	0.0001	0.6005	0.0582	0.0000	0.0606	0.1810	0.8716	0.7248	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0539	0.0805	0.7605	0.1939
6	M	B	{65}	0.0000	0.9990	0.9803	0.9667	0.9951	0.0000	0.9846	0.9981	0.8405	0.9426	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9630	0.9992	0.9306	0.9993
6	P	A	{66}	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
6	P	3	{67}	0.0000	0.9975	0.9739	0.9654	0.9974	0.0000	0.9925	0.9984	0.8252	0.9384	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9840	0.9994	0.9250	0.9994
6	P	2	{68}	0.0001	0.3699	0.0661	0.9979	0.8778	0.0000	0.8908	0.9604	0.9247	0.9981	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.8824	0.8969	0.9980	0.9454
6	P	1	{69}	0.0000	0.0287	0.0018	0.9289	0.3087	0.0000	0.3195	0.5797	0.9841	0.9634	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.2985	0.3706	0.9707	0.5865
6	P	B	{70}	0.0000	0.9990	0.9573	0.9887	0.9969	0.0000	0.9998	0.9957	0.9747	0.9878	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.9519	0.9865	0.9998
6	N	A	{71}	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6	N	3	{72}	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000

6	N	2	{73}	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000
6	N	1	{74}	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.0000
6	N	B	{75}	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
6	C	A	{76}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0001
6	C	3	{77}	0.0000	0.9946	0.9364	0.9953	0.9991	0.0000	0.9899	0.9999	0.9667	0.9913	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9279	1.0000	0.9889	1.0000
6	C	2	{78}	0.0000	0.0460	0.0033	0.9482	0.3897	0.0000	0.4033	0.6554	0.9803	0.9716	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.3814	0.4516	0.9764	0.6531
6	C	1	{79}	0.0000	0.0140	0.0007	0.8676	0.2037	0.0000	0.2113	0.4466	0.9716	0.9272	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1944	0.2556	0.9409	0.4591
6	C	B	{80}	0.0000	0.9947	0.8883	0.9953	0.9957	0.0000	0.9990	0.9969	0.9945	0.9965	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9996	0.8094	0.9963	1.0000
8	M	A	{81}	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
8	M	3	{82}	0.0000	0.0412	0.0899	0.0002	0.0098	0.0000	0.0088	0.0027	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0092	0.0078	0.0001	0.0025
8	M	2	{83}	0.0000	0.0455	0.0033	0.9533	0.3925	0.0000	0.4058	0.6632	0.9862	0.9759	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.3833	0.4564	0.9804	0.6636
8	M	1	{84}	0.0000	0.7425	0.2691	0.9976	0.9785	0.0001	0.9837	0.9890	0.9999	0.9722	0.0000	0.0001	0.0001	0.0000	0.0000	0.0001	0.9833	0.9763	0.8802	0.9657
8	M	B	{85}	0.0000	0.9030	0.4928	0.9485	0.9934	0.0000	0.9959	0.9899	0.9998	0.9857	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.9962	0.9884	0.9953	0.9093
8	P	A	{86}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0001
8	P	3	{87}	0.0000	0.0947	0.1297	0.0011	0.0375	0.0000	0.0334	0.0131	0.0001	0.0005	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0344	0.0320	0.0003	0.0124
8	P	2	{88}	0.0000	0.0401	0.0027	0.9497	0.3699	0.0000	0.3824	0.6444	0.9878	0.9747	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.3599	0.4345	0.9798	0.6476
8	P	1	{89}	0.0001	0.3264	0.0535	0.9976	0.8537	0.0000	0.8676	0.9525	0.9692	0.9982	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.8572	0.8778	0.9981	0.9382
8	P	B	{90}	0.0001	0.6342	0.1810	0.9996	0.9682	0.0001	0.9741	0.9907	1.0000	0.9994	0.0000	0.0001	0.0000	0.0000	0.0000	0.0001	0.9720	0.9716	0.9990	0.9815
8	N	A	{91}	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.0000
8	N	3	{92}	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000
8	N	2	{93}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000
8	N	1	{94}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000
8	N	B	{95}	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.0000
8	C	A	{96}	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
8	C	3	{97}	0.0000	0.1737	0.1242	0.0057	0.1114	0.0000	0.0998	0.0494	0.0005	0.0027	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1005	0.1016	0.0020	0.0483
8	C	2	{98}	0.0000	0.0442	0.0032	0.9383	0.3759	0.0000	0.3897	0.6357	0.9692	0.9638	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.3686	0.4351	0.9691	0.6306
8	C	1	{99}	0.0001	0.4764	0.1026	0.9991	0.9272	0.0001	0.9369	0.9785	0.8211	0.9990	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.9317	0.9383	0.9989	0.9667
8	C	B	{100}	0.0000	0.9032	0.5054	1.0000	0.9890	0.0000	0.9934	0.9612	0.9999	0.9994	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9943	0.9753	0.9997	0.5465

(Cont.)

				{41}	{42}	{43}	{44}	{45}	{46}	{47}	{48}	{49}	{50}	{51}	{52}	{53}	{54}	{55}	{56}	{57}	{58}	{59}	{60}
Week	Treatments	Position		30.63	6.81	2.94	2.50	3.00	30.43	4.33	2.80	1.10	2.00	31.00	29.67	32.00	29.67	30.33	29.50	7.20	3.80	2.17	2.67
0	M	A	{1}	0.0094	0.0000	0.0001	0.0001	0.0001	0.0166	0.0000	0.0001	0.0000	0.0000	0.0026	0.1085	0.0001	0.1209	0.0203	0.1439	0.0000	0.0000	0.0000	0.0001
0	M	3	{2}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000

0	M	2	{3}	0.0000	0.9921	0.2874	0.1115	0.3150	0.0000	0.9409	0.2205	0.0011	0.0339	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9697	0.7902	0.0524	0.1662
0	M	1	{4}	0.0001	0.9977	0.8429	0.6424	0.8524	0.0001	0.9622	0.7910	0.0576	0.4466	0.0001	0.0000	0.0001	0.0000	0.0001	0.0000	0.9757	0.9779	0.5205	0.7323
0	M	B	{5}	0.0000	0.9996	0.4019	0.1810	0.4313	0.0000	0.9604	0.3231	0.0027	0.0655	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9949	0.8629	0.0956	0.2549
0	P	A	{6}	0.0939	0.0000	0.0000	0.0000	0.0000	0.1310	0.0000	0.0000	0.0000	0.0000	0.0389	0.3603	0.0014	0.4150	0.1401	0.3924	0.0000	0.0001	0.0000	0.0000
0	P	3	{7}	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0001
0	P	2	{8}	0.0000	0.9569	0.2978	0.1160	0.3264	0.0000	0.9497	0.2288	0.0011	0.0352	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9214	0.8050	0.0544	0.1727
0	P	1	{9}	0.0001	0.9967	0.8618	0.6648	0.8716	0.0001	0.9820	0.8117	0.0605	0.4616	0.0001	0.0000	0.0001	0.0000	0.0000	0.0000	0.9698	0.9852	0.5380	0.7541
0	P	B	{10}	0.0000	0.9992	0.6820	0.4158	0.7059	0.0000	0.9878	0.6015	0.0152	0.2113	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9901	0.9626	0.2751	0.5205
0	N	A	{11}	0.0177	0.0000	0.0001	0.0000	0.0001	0.0294	0.0000	0.0001	0.0000	0.0000	0.0054	0.1539	0.0001	0.1735	0.0347	0.1937	0.0000	0.0000	0.0000	0.0001
0	N	3	{12}	0.0630	0.0000	0.0001	0.0000	0.0001	0.0935	0.0000	0.0000	0.0000	0.0000	0.0236	0.3185	0.0007	0.3587	0.1041	0.3661	0.0000	0.0001	0.0000	0.0000
0	N	2	{13}	0.0556	0.0000	0.0000	0.0000	0.0001	0.0820	0.0000	0.0000	0.0000	0.0000	0.0208	0.2772	0.0006	0.3185	0.0904	0.3154	0.0000	0.0001	0.0000	0.0000
0	N	1	{14}	0.0002	0.0000	0.0001	0.0001	0.0001	0.0005	0.0000	0.0001	0.0000	0.0000	0.0001	0.0066	0.0000	0.0074	0.0006	0.0103	0.0000	0.0000	0.0000	0.0001
0	N	B	{15}	0.0002	0.0000	0.0001	0.0001	0.0001	0.0004	0.0000	0.0001	0.0000	0.0000	0.0001	0.0054	0.0000	0.0061	0.0005	0.0087	0.0000	0.0000	0.0000	0.0001
0	C	A	{16}	0.0814	0.0000	0.0000	0.0000	0.0000	0.1126	0.0000	0.0000	0.0000	0.0000	0.0338	0.3032	0.0012	0.3603	0.1186	0.3223	0.0000	0.0001	0.0000	0.0000
0	C	3	{17}	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000
0	C	2	{18}	0.0000	0.9959	0.3392	0.1401	0.3686	0.0000	0.9557	0.2655	0.0016	0.0456	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9791	0.8318	0.0689	0.2040
0	C	1	{19}	0.0000	0.7503	0.9892	0.9714	0.9857	0.0000	0.9997	0.9862	0.5570	0.9682	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.4835	0.8565	0.9740	0.9820
0	C	B	{20}	0.0000	0.9963	0.8572	0.6476	0.8694	0.0000	0.9928	0.8034	0.0515	0.4288	0.0001	0.0000	0.0001	0.0000	0.0000	0.0000	0.9691	0.9886	0.5085	0.7416
2	M	A	{21}	0.0346	0.0000	0.0001	0.0000	0.0001	0.0543	0.0000	0.0001	0.0000	0.0000	0.0117	0.2291	0.0003	0.2583	0.0623	0.2752	0.0000	0.0000	0.0000	0.0000
2	M	3	{22}	0.0000	0.9549	0.1751	0.0582	0.1961	0.0000	0.8778	0.1277	0.0004	0.0151	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9128	0.6546	0.0246	0.0919
2	M	2	{23}	0.0000	0.8082	0.0207	0.0046	0.0246	0.0000	0.4420	0.0132	0.0001	0.0008	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.7862	0.1956	0.0015	0.0084
2	M	1	{24}	0.0001	0.9063	0.9904	0.9525	0.9907	0.0001	0.9876	0.9838	0.3293	0.8917	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.7198	0.9994	0.9213	0.9740
2	M	B	{25}	0.0000	0.9984	0.7004	0.4313	0.7248	0.0000	0.9920	0.6199	0.0158	0.2189	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9852	0.9698	0.2851	0.5380
2	P	A	{26}	0.3414	0.0000	0.0000	0.0000	0.0000	0.4078	0.0000	0.0000	0.0000	0.0000	0.1937	0.6595	0.0156	0.7406	0.4080	0.6555	0.0000	0.0001	0.0000	0.0000
2	P	3	{27}	0.0000	0.9970	0.7180	0.4466	0.7425	0.0000	0.9947	0.6376	0.0165	0.2265	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9779	0.9757	0.2951	0.5550
2	P	2	{28}	0.0001	0.9953	0.8785	0.6859	0.8885	0.0000	0.9915	0.8305	0.0635	0.4764	0.0001	0.0000	0.0001	0.0000	0.0000	0.0000	0.9626	0.9901	0.5550	0.7742
2	P	1	{29}	0.0000	0.6188	0.9776	0.9697	0.9612	0.0000	0.9999	0.9784	0.6640	0.9837	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3474	0.9989	0.9846	0.9774
2	P	B	{30}	0.0000	0.8369	0.9938	0.9716	0.9934	0.0001	0.9399	0.9901	0.4539	0.9454	0.0000	0.0001	0.0000	0.0001	0.0001	0.0001	0.6015	0.9986	0.9600	0.9846
2	N	A	{31}	0.0085	0.0000	0.0001	0.0001	0.0001	0.0148	0.0000	0.0001	0.0000	0.0000	0.0023	0.0964	0.0001	0.1085	0.0180	0.1272	0.0000	0.0000	0.0000	0.0001
2	N	3	{32}	0.3839	0.0000	0.0000	0.0000	0.0000	0.4337	0.0001	0.0000	0.0000	0.0000	0.2380	0.5749	0.0243	0.7028	0.4119	0.4952	0.0000	0.0001	0.0000	0.0000
2	N	2	{33}	0.3244	0.0000	0.0000	0.0000	0.0000	0.3595	0.0001	0.0000	0.0000	0.0000	0.2007	0.4058	0.0200	0.5749	0.3258	0.2583	0.0000	0.0001	0.0000	0.0000
2	N	1	{34}	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
2	N	B	{35}	0.0001	0.0000	0.0001	0.0001	0.0000	0.0002	0.0000	0.0001	0.0000	0.0000	0.0000	0.0029	0.0000	0.0032	0.0003	0.0048	0.0000	0.0000	0.0000	0.0001
2	C	A	{36}	0.2982	0.0000	0.0000	0.0000	0.0000	0.3537	0.0000	0.0000	0.0000	0.0000	0.1681	0.5576	0.0133	0.6595	0.3464	0.5267	0.0000	0.0001	0.0000	0.0000

2	C	3	{37}	0.0000	0.9960	0.7004	0.4251	0.7264	0.0000	0.9950	0.6177	0.0144	0.2083	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9744	0.9741	0.2746	0.5336
2	C	2	{38}	0.0000	0.9990	0.7443	0.4903	0.7640	0.0000	0.9878	0.6713	0.0235	0.2752	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9878	0.9719	0.3462	0.5947
2	C	1	{39}	0.0000	0.8065	0.9943	0.9754	0.9936	0.0000	0.9876	0.9911	0.4962	0.9572	0.0000	0.0001	0.0000	0.0001	0.0001	0.0001	0.5579	0.9974	0.9682	0.9865
2	C	B	{40}	0.0001	0.9968	0.8620	0.6770	0.8694	0.0001	0.9436	0.8153	0.0703	0.4924	0.0001	0.0000	0.0001	0.0001	0.0001	0.0000	0.9690	0.9785	0.5644	0.7616
4	M	A	{41}		0.0000	0.0000	0.0000	0.0000	0.8590	0.0001	0.0000	0.0000	0.0000	0.7345	0.9091	0.4275	0.8220	0.9624	0.9123	0.0000	0.0000	0.0000	0.0000
4	M	3	{42}	0.0000		0.1554	0.0498	0.1751	0.0000	0.8636	0.1121	0.0003	0.0124	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.7260	0.6249	0.0205	0.0797
4	M	2	{43}	0.0000	0.1554		0.9790	0.9549	0.0000	0.9930	0.9010	0.9444	0.9998	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0537	0.9941	0.9995	0.9675
4	M	1	{44}	0.0000	0.0498	0.9790		0.9914	0.0000	0.9667	0.9602	0.9832	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0138	0.9851	0.9999	0.8802
4	M	B	{45}	0.0000	0.1751	0.9549	0.9914		0.0000	0.9928	0.9821	0.9424	0.9998	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0624	0.9912	0.9996	0.9905
4	P	A	{46}	0.8590	0.0000	0.0000	0.0000	0.0000		0.0001	0.0000	0.0000	0.0000	0.8631	0.9013	0.4860	0.7699	0.9314	0.9182	0.0000	0.0000	0.0000	0.0000
4	P	3	{47}	0.0001	0.8636	0.9930	0.9667	0.9928	0.0001		0.9886	0.4116	0.9308	0.0000	0.0001	0.0000	0.0001	0.0001	0.0001	0.6434	0.9991	0.9497	0.9819
4	P	2	{48}	0.0000	0.1121	0.9010	0.9602	0.9821	0.0000	0.9886		0.9609	0.9999	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0360	0.9928	0.9997	0.9040
4	P	1	{49}	0.0000	0.0003	0.9444	0.9832	0.9424	0.0000	0.4116	0.9609		0.6943	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.6773	0.9291	0.9720
4	P	B	{50}	0.0000	0.0124	0.9998	1.0000	0.9998	0.0000	0.9308	0.9999	0.6943		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0028	0.9872	0.9988	1.0000
4	N	A	{51}	0.7345	0.0000	0.0000	0.0000	0.0000	0.8631	0.0000	0.0000	0.0000	0.0000		0.8342	0.3658	0.7480	0.9312	0.8248	0.0000	0.0000	0.0000	0.0000
4	N	3	{52}	0.9091	0.0000	0.0000	0.0000	0.0000	0.9013	0.0001	0.0000	0.0000	0.0000	0.8342		0.3464	1.0000	0.8185	0.8802	0.0000	0.0000	0.0000	0.0000
4	N	2	{53}	0.4275	0.0000	0.0000	0.0000	0.0000	0.4860	0.0000	0.0000	0.0000	0.0000	0.3658	0.3464		0.2818	0.5576	0.3154	0.0000	0.0000	0.0000	0.0000
4	N	1	{54}	0.8220	0.0000	0.0000	0.0000	0.0000	0.7699	0.0001	0.0000	0.0000	0.0000	0.7480	1.0000	0.2818		0.5465	0.9876	0.0000	0.0000	0.0000	0.0000
4	N	B	{55}	0.9624	0.0000	0.0000	0.0000	0.0000	0.9314	0.0001	0.0000	0.0000	0.0000	0.9312	0.8185	0.5576	0.5465		0.8751	0.0000	0.0000	0.0000	0.0000
4	C	A	{56}	0.9123	0.0000	0.0000	0.0000	0.0000	0.9182	0.0001	0.0000	0.0000	0.0000	0.8248	0.8802	0.3154	0.9876	0.8751		0.0000	0.0000	0.0000	0.0000
4	C	3	{57}	0.0000	0.7260	0.0537	0.0138	0.0624	0.0000	0.6434	0.0360	0.0001	0.0028	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.3556	0.0049	0.0239
4	C	2	{58}	0.0000	0.6249	0.9941	0.9851	0.9912	0.0000	0.9991	0.9928	0.6773	0.9872	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3556		0.9892	0.9908
4	C	1	{59}	0.0000	0.0205	0.9995	0.9999	0.9996	0.0000	0.9497	0.9997	0.9291	0.9988	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0049	0.9892		0.9998
4	C	B	{60}	0.0000	0.0797	0.9675	0.8802	0.9905	0.0000	0.9819	0.9040	0.9720	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0239	0.9908	0.9998	
6	M	A	{61}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6	M	3	{62}	0.0000	0.7346	0.9943	0.9803	0.9928	0.0000	0.9982	0.9920	0.5786	0.9741	0.0000	0.0001	0.0000	0.0000	0.0000	0.0001	0.4677	0.9821	0.9798	0.9886
6	M	2	{63}	0.0000	0.0120	0.9995	1.0000	0.9996	0.0000	0.9213	0.9997	0.8479	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0027	0.9833	0.9876	0.9999
6	M	1	{64}	0.0000	0.0017	0.9908	0.9982	0.9907	0.0000	0.6859	0.9943	0.7175	0.6511	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0003	0.8837	0.9747	0.9963
6	M	B	{65}	0.0000	0.9995	0.3584	0.1534	0.3874	0.0000	0.9525	0.2836	0.0020	0.0524	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9940	0.8370	0.0780	0.2203
6	P	A	{66}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6	P	3	{67}	0.0000	0.9989	0.3344	0.1385	0.3631	0.0000	0.9494	0.2619	0.0016	0.0455	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9905	0.8229	0.0686	0.2014
6	P	2	{68}	0.0000	0.3399	0.9843	0.9891	0.9511	0.0000	0.9982	0.9890	0.8601	0.9982	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1467	0.9934	0.9979	0.9909
6	P	1	{69}	0.0000	0.0240	0.9993	0.9999	0.9995	0.0000	0.9542	0.9996	0.9508	0.9996	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0059	0.9894	0.9599	0.9997
6	P	B	{70}	0.0000	0.9990	0.7200	0.4591	0.7416	0.0000	0.9886	0.6434	0.0195	0.2467	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9886	0.9691	0.3150	0.5644

6	N	A	{71}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6	N	3	{72}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6	N	2	{73}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6	N	1	{74}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6	N	B	{75}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6	C	A	{76}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6	C	3	{77}	0.0000	0.9961	0.6454	0.3686	0.6739	0.0000	0.9931	0.5589	0.0103	0.1685	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9761	0.9637	0.2275	0.4740
6	C	2	{78}	0.0000	0.0391	0.9967	0.9955	0.9982	0.0000	0.9655	0.9963	0.9761	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0103	0.9890	0.9996	0.9951
6	C	1	{79}	0.0000	0.0115	0.9990	0.9998	0.9991	0.0000	0.9106	0.9994	0.9264	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0026	0.9783	0.8802	0.9996
6	C	B	{80}	0.0000	0.9945	0.8727	0.6673	0.8848	0.0000	0.9964	0.8209	0.0539	0.4423	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.9608	0.9921	0.5242	0.7605
8	M	A	{81}	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
8	M	3	{82}	0.0000	0.0366	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0689	0.0000	0.0001	0.0000
8	M	2	{83}	0.0000	0.0386	0.9989	0.9996	0.9993	0.0000	0.9700	0.9990	0.9643	0.9999	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0102	0.9921	0.9973	0.9991
8	M	1	{84}	0.0000	0.7180	0.9970	0.9865	0.9964	0.0000	0.9905	0.9953	0.5993	0.9788	0.0000	0.0001	0.0000	0.0001	0.0000	0.0001	0.4516	0.9979	0.9843	0.9928
8	M	B	{85}	0.0001	0.8915	0.9913	0.9580	0.9913	0.0001	0.9314	0.9855	0.3619	0.9087	0.0001	0.0001	0.0000	0.0001	0.0001	0.0001	0.6912	0.9992	0.9336	0.9770
8	P	A	{86}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
8	P	3	{87}	0.0000	0.0791	0.0000	0.0000	0.0000	0.0000	0.0006	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1222	0.0001	0.0001	0.0000
8	P	2	{88}	0.0000	0.0338	0.9994	0.9999	0.9996	0.0000	0.9682	0.9996	0.9537	0.9997	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0087	0.9926	0.9876	0.9997
8	P	1	{89}	0.0000	0.2978	0.9569	0.9844	0.8211	0.0000	0.9981	0.9772	0.8764	0.9986	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1227	0.9963	0.9981	0.9846
8	P	B	{90}	0.0000	0.6033	0.9904	0.9817	0.9844	0.0000	0.9995	0.9895	0.6883	0.9877	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3353	0.9639	0.9890	0.9877
8	N	A	{91}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
8	N	3	{92}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
8	N	2	{93}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
8	N	1	{94}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
8	N	B	{95}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
8	C	A	{96}	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000
8	C	3	{97}	0.0000	0.1348	0.0000	0.0000	0.0001	0.0000	0.0035	0.0000	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1666	0.0005	0.0001	0.0000
8	C	2	{98}	0.0000	0.0375	0.9887	0.9279	0.9944	0.0000	0.9571	0.9838	0.9851	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0099	0.9832	0.9999	0.9685
8	C	1	{99}	0.0000	0.4441	0.9865	0.9857	0.9692	0.0000	0.9991	0.9886	0.7971	0.9957	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2122	0.9930	0.9955	0.9891
8	C	B	{100}	0.0001	0.8932	0.9933	0.9611	0.9937	0.0001	0.9988	0.9880	0.3425	0.9021	0.0001	0.0001	0.0001	0.0001	0.0001	0.0000	0.6992	0.9998	0.9308	0.9798

(Cont.)

				{61}	{62}	{63}	{64}	{65}	{66}	{67}	{68}	{69}	{70}	{71}	{72}	{73}	{74}	{75}	{76}	{77}	{78}	{79}	{80}
Week	Treatments	Position		42.67	4.00	2.00	1.50	6.33	43.00	6.39	3.33	2.22	5.67	45.50	45.00	44.50	43.33	43.00	42.00	5.90	2.40	2.00	5.33
0	M	A	{1}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

0	M	3	{2}	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000
0	M	2	{3}	0.0000	0.8676	0.0327	0.0056	0.9988	0.0000	0.9945	0.5242	0.0601	0.9996	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.9944	0.0911	0.0315	0.9979
0	M	1	{4}	0.0001	0.9817	0.4313	0.1659	0.9995	0.0000	0.9995	0.9382	0.5437	0.9998	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	1.0000	0.6150	0.4158	1.0000
0	M	B	{5}	0.0000	0.9160	0.0630	0.0125	0.9999	0.0000	0.9913	0.6444	0.1075	0.9985	0.0001	0.0001	0.0001	0.0000	0.0000	0.0000	0.7516	0.1532	0.0606	0.9960
0	P	A	{6}	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0	P	3	{7}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
0	P	2	{8}	0.0000	0.8802	0.0339	0.0058	0.9999	0.0000	0.9996	0.5395	0.0624	0.9998	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.9982	0.0947	0.0327	0.9988
0	P	1	{9}	0.0001	0.9890	0.4466	0.1734	0.9990	0.0001	0.9991	0.9505	0.5620	0.9990	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	1.0000	0.6357	0.4313	0.9998
0	P	B	{10}	0.0001	0.9785	0.2037	0.0558	0.9985	0.0001	0.9991	0.8633	0.2978	0.9399	0.0000	0.0001	0.0001	0.0001	0.0001	0.0000	0.9999	0.3759	0.1961	0.9818
0	N	A	{11}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0	N	3	{12}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0	N	2	{13}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0	N	1	{14}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
0	N	B	{15}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
0	C	A	{16}	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0	C	3	{17}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000
0	C	2	{18}	0.0000	0.8975	0.0439	0.0080	0.9980	0.0000	0.9920	0.5841	0.0784	0.9997	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.9914	0.1160	0.0423	0.9984
0	C	1	{19}	0.0000	1.0000	0.9611	0.8035	0.9106	0.0000	0.9017	0.9909	0.9750	0.9867	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9855	0.9763	0.9525	0.9972
0	C	B	{20}	0.0001	0.9928	0.4152	0.1534	0.9982	0.0001	0.9985	0.9525	0.5341	0.9905	0.0000	0.0000	0.0000	0.0001	0.0001	0.0001	0.9999	0.6138	0.4014	1.0000
2	M	A	{21}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
2	M	3	{22}	0.0000	0.7592	0.0146	0.0022	0.9990	0.0000	0.9975	0.3699	0.0287	0.9990	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.9946	0.0460	0.0140	0.9947
2	M	2	{23}	0.0000	0.2807	0.0008	0.0001	0.9803	0.0000	0.9739	0.0661	0.0018	0.9573	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9364	0.0033	0.0007	0.8883
2	M	1	{24}	0.0000	0.9994	0.8802	0.6005	0.9667	0.0000	0.9654	0.9979	0.9289	0.9887	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9953	0.9482	0.8676	0.9953
2	M	B	{25}	0.0000	0.9837	0.2113	0.0582	0.9951	0.0001	0.9974	0.8778	0.3087	0.9969	0.0000	0.0001	0.0001	0.0001	0.0001	0.0000	0.9991	0.3897	0.2037	0.9957
2	P	A	{26}	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
2	P	3	{27}	0.0000	0.9877	0.2189	0.0606	0.9846	0.0000	0.9925	0.8908	0.3195	0.9998	0.0000	0.0001	0.0001	0.0001	0.0001	0.0000	0.9899	0.4033	0.2113	0.9990
2	P	2	{28}	0.0001	0.9934	0.4616	0.1810	0.9981	0.0001	0.9984	0.9604	0.5797	0.9957	0.0000	0.0000	0.0000	0.0000	0.0001	0.0001	0.9999	0.6554	0.4466	0.9969
2	P	1	{29}	0.0000	0.9994	0.9785	0.8716	0.8405	0.0000	0.8252	0.9247	0.9841	0.9747	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9667	0.9803	0.9716	0.9945
2	P	B	{30}	0.0000	0.9959	0.9369	0.7248	0.9426	0.0000	0.9384	0.9981	0.9634	0.9878	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9913	0.9716	0.9272	0.9965
2	N	A	{31}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
2	N	3	{32}	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

2	N	2	{33}	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
2	N	1	{34}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
2	N	B	{35}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
2	C	A	{36}	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
2	C	3	{37}	0.0000	0.9872	0.2013	0.0539	0.9630	0.0000	0.9840	0.8824	0.2985	1.0000	0.0000	0.0001	0.0001	0.0001	0.0000	0.0000	0.9279	0.3814	0.1944	0.9996
2	C	2	{38}	0.0001	0.9830	0.2655	0.0805	0.9992	0.0001	0.9994	0.8969	0.3706	0.9519	0.0000	0.0000	0.0000	0.0001	0.0001	0.0001	1.0000	0.4516	0.2556	0.8094
2	C	1	{39}	0.0000	0.9876	0.9497	0.7605	0.9306	0.0000	0.9250	0.9980	0.9707	0.9865	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9889	0.9764	0.9409	0.9963
2	C	B	{40}	0.0000	0.9803	0.4760	0.1939	0.9993	0.0000	0.9994	0.9454	0.5865	0.9998	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	1.0000	0.6531	0.4591	1.0000
4	M	A	{41}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
4	M	3	{42}	0.0000	0.7346	0.0120	0.0017	0.9995	0.0000	0.9989	0.3399	0.0240	0.9990	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9961	0.0391	0.0115	0.9945
4	M	2	{43}	0.0000	0.9943	0.9995	0.9908	0.3584	0.0000	0.3344	0.9843	0.9993	0.7200	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.6454	0.9967	0.9990	0.8727
4	M	1	{44}	0.0000	0.9803	1.0000	0.9982	0.1534	0.0000	0.1385	0.9891	0.9999	0.4591	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3686	0.9955	0.9998	0.6673
4	M	B	{45}	0.0000	0.9928	0.9996	0.9907	0.3874	0.0000	0.3631	0.9511	0.9995	0.7416	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.6739	0.9982	0.9991	0.8848
4	P	A	{46}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
4	P	3	{47}	0.0000	0.9982	0.9213	0.6859	0.9525	0.0000	0.9494	0.9982	0.9542	0.9886	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9931	0.9655	0.9106	0.9964
4	P	2	{48}	0.0000	0.9920	0.9997	0.9943	0.2836	0.0000	0.2619	0.9890	0.9996	0.6434	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.5589	0.9963	0.9994	0.8209
4	P	1	{49}	0.0000	0.5786	0.8479	0.7175	0.0020	0.0000	0.0016	0.8601	0.9508	0.0195	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0103	0.9761	0.9264	0.0539
4	P	B	{50}	0.0000	0.9741	1.0000	0.6511	0.0524	0.0000	0.0455	0.9982	0.9996	0.2467	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1685	1.0000	1.0000	0.4423
4	N	A	{51}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
4	N	3	{52}	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
4	N	2	{53}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
4	N	1	{54}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
4	N	B	{55}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
4	C	A	{56}	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
4	C	3	{57}	0.0000	0.4677	0.0027	0.0003	0.9940	0.0000	0.9905	0.1467	0.0059	0.9886	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9761	0.0103	0.0026	0.9608
4	C	2	{58}	0.0000	0.9821	0.9833	0.8837	0.8370	0.0000	0.8229	0.9934	0.9894	0.9691	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9637	0.9890	0.9783	0.9921
4	C	1	{59}	0.0000	0.9798	0.9876	0.9747	0.0780	0.0000	0.0686	0.9979	0.9599	0.3150	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2275	0.9996	0.8802	0.5242
4	C	B	{60}	0.0000	0.9886	0.9999	0.9963	0.2203	0.0000	0.2014	0.9909	0.9997	0.5644	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.4740	0.9951	0.9996	0.7605
6	M	A	{61}		0.0000	0.0000	0.0000	0.0000	0.7631	0.0000	0.0000	0.0000	0.0001	0.3012	0.4080	0.6442	0.9312	0.9511	0.5465	0.0000	0.0000	0.0000	0.0001
6	M	3	{62}	0.0000		0.9682	0.8218	0.8985	0.0000	0.8895	0.9967	0.9809	0.9819	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9817	0.9830	0.9611	0.9955
6	M	2	{63}	0.0000	0.9682		0.8934	0.0505	0.0000	0.0439	0.9972	0.9971	0.2379	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1628	0.9998	1.0000	0.4288

6	M	1	{64}	0.0000	0.8218	0.8934		0.0095	0.0000	0.0080	0.9667	0.9868	0.0690	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0407	0.9965	0.9692	0.1598
6	M	B	{65}	0.0000	0.8985	0.0505	0.0095		0.0000	0.9599	0.6005	0.0882	0.9989	0.0001	0.0001	0.0001	0.0000	0.0000	0.0000	0.9188	0.1283	0.0485	0.9964
6	P	A	{66}	0.7631	0.0000	0.0000	0.0000	0.0000		0.0000	0.0000	0.0000	0.0001	0.4610	0.5420	0.7530	0.9511	1.0000	0.6375	0.0000	0.0000	0.0000	0.0001
6	P	3	{67}	0.0000	0.8895	0.0439	0.0080	0.9599	0.0000		0.5760	0.0780	0.9993	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.9711	0.1150	0.0422	0.9971
6	P	2	{68}	0.0000	0.9967	0.9972	0.9667	0.6005	0.0000	0.5760		0.9976	0.8848	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.8490	0.9955	0.9955	0.9611
6	P	1	{69}	0.0000	0.9809	0.9971	0.9868	0.0882	0.0000	0.0780	0.9976		0.3389	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2492	0.9985	0.9780	0.5505
6	P	B	{70}	0.0001	0.9819	0.2379	0.0690	0.9989	0.0001	0.9993	0.8848	0.3389		0.0000	0.0000	0.0001	0.0001	0.0001	0.0001	0.9999	0.4194	0.2291	0.9511
6	N	A	{71}	0.3012	0.0000	0.0000	0.0000	0.0001	0.4610	0.0001	0.0000	0.0000	0.0000		0.9914	0.9455	0.5718	0.4148	0.0778	0.0001	0.0000	0.0000	0.0000
6	N	3	{72}	0.4080	0.0000	0.0000	0.0000	0.0001	0.5420	0.0001	0.0000	0.0000	0.0000	0.9914		0.6511	0.5576	0.4599	0.1430	0.0001	0.0000	0.0000	0.0000
6	N	2	{73}	0.6442	0.0000	0.0000	0.0000	0.0001	0.7530	0.0000	0.0000	0.0000	0.0001	0.9455	0.6511		0.7168	0.6555	0.3154	0.0001	0.0000	0.0000	0.0000
6	N	1	{74}	0.9312	0.0000	0.0000	0.0000	0.0000	0.9511	0.0000	0.0000	0.0000	0.0001	0.5718	0.5576	0.7168		0.7631	0.7480	0.0000	0.0000	0.0000	0.0001
6	N	B	{75}	0.9511	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	0.0000	0.0000	0.0001	0.4148	0.4599	0.6555	0.7631		0.8025	0.0000	0.0000	0.0000	0.0001
6	C	A	{76}	0.5465	0.0000	0.0000	0.0000	0.0000	0.6375	0.0000	0.0000	0.0000	0.0001	0.0778	0.1430	0.3154	0.7480	0.8025		0.0000	0.0000	0.0000	0.0001
6	C	3	{77}	0.0000	0.9817	0.1628	0.0407	0.9188	0.0000	0.9711	0.8490	0.2492	0.9999	0.0001	0.0001	0.0001	0.0000	0.0000	0.0000		0.3264	0.1571	0.9996
6	C	2	{78}	0.0000	0.9830	0.9998	0.9965	0.1283	0.0000	0.1150	0.9955	0.9985	0.4194	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3264		0.9992	0.6322
6	C	1	{79}	0.0000	0.9611	1.0000	0.9692	0.0485	0.0000	0.0422	0.9955	0.9780	0.2291	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1571	0.9992		0.4152
6	C	B	{80}	0.0001	0.9955	0.4288	0.1598	0.9964	0.0001	0.9971	0.9611	0.5505	0.9511	0.0000	0.0000	0.0000	0.0001	0.0001	0.0001	0.9996	0.6322	0.4152	
8	M	A	{81}	0.0043	0.0000	0.0000	0.0000	0.0001	0.0116	0.0001	0.0000	0.0000	0.0000	0.1502	0.4076	0.2234	0.0237	0.0099	0.0004	0.0000	0.0000	0.0000	0.0000
8	M	3	{82}	0.0000	0.0000	0.0001	0.0000	0.0338	0.0000	0.0340	0.0000	0.0001	0.0090	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0114	0.0001	0.0001	0.0032
8	M	2	{83}	0.0000	0.9866	0.9993	0.9932	0.1282	0.0000	0.1147	0.9976	0.9882	0.4235	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3275	0.9889	0.9969	0.6385
8	M	1	{84}	0.0000	1.0000	0.9741	0.8385	0.8848	0.0000	0.8758	0.9989	0.9854	0.9754	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9769	0.9878	0.9682	0.9928
8	M	B	{85}	0.0000	0.9989	0.8980	0.6357	0.9626	0.0000	0.9606	0.9979	0.9397	0.9897	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9948	0.9554	0.8859	0.9964
8	P	A	{86}	0.0661	0.0000	0.0000	0.0000	0.0000	0.0551	0.0000	0.0000	0.0000	0.0000	0.0002	0.0006	0.0032	0.0523	0.0836	0.1035	0.0000	0.0000	0.0000	0.0001
8	P	3	{87}	0.0000	0.0002	0.0001	0.0001	0.0974	0.0000	0.0955	0.0000	0.0001	0.0357	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0405	0.0000	0.0001	0.0147
8	P	2	{88}	0.0000	0.9867	0.9982	0.9891	0.1160	0.0000	0.1034	0.9982	0.9200	0.4014	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3054	0.9980	0.9905	0.6177
8	P	1	{89}	0.0000	0.9976	0.9976	0.9716	0.5550	0.0000	0.5294	0.9399	0.9977	0.8633	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.8191	0.9946	0.9960	0.9521
8	P	B	{90}	0.0000	0.9959	0.9837	0.8885	0.8259	0.0000	0.8105	0.9818	0.9890	0.9683	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9606	0.9874	0.9785	0.9922
8	N	A	{91}	0.5183	0.0000	0.0000	0.0000	0.0000	0.5845	0.0000	0.0000	0.0000	0.0001	0.9753	0.9485	0.9955	0.3347	0.4144	0.2516	0.0001	0.0000	0.0000	0.0001
8	N	3	{92}	0.3200	0.0000	0.0000	0.0000	0.0001	0.4580	0.0001	0.0000	0.0000	0.0000	0.9959	0.8211	0.7761	0.5094	0.3924	0.0949	0.0001	0.0000	0.0000	0.0000
8	N	2	{93}	0.3652	0.0000	0.0000	0.0000	0.0001	0.5186	0.0001	0.0000	0.0000	0.0000	0.9722	0.9722	0.9054	0.5934	0.4580	0.1108	0.0001	0.0000	0.0000	0.0000
8	N	1	{94}	0.3055	0.0000	0.0000	0.0000	0.0001	0.4591	0.0001	0.0000	0.0000	0.0000	0.9485	0.9802	0.9182	0.5543	0.4078	0.0820	0.0001	0.0000	0.0000	0.0000

8	N	B	{95}	0.6200	0.0000	0.0000	0.0000	0.0000	0.7120	0.0000	0.0000	0.0000	0.0001	0.9553	0.8502	0.9279	0.5992	0.5845	0.3116	0.0001	0.0000	0.0000	0.0000
8	C	A	{96}	0.0015	0.0000	0.0000	0.0000	0.0001	0.0047	0.0001	0.0000	0.0000	0.0000	0.1982	0.3116	0.1476	0.0107	0.0040	0.0001	0.0000	0.0000	0.0000	0.0000
8	C	3	{97}	0.0000	0.0011	0.0001	0.0001	0.2209	0.0000	0.2117	0.0001	0.0001	0.1101	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1129	0.0000	0.0001	0.0533
8	C	2	{98}	0.0000	0.9763	1.0000	0.9984	0.1231	0.0000	0.1104	0.9905	0.9999	0.4041	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3150	1.0000	0.9998	0.6138
8	C	1	{99}	0.0000	0.9976	0.9937	0.9419	0.7037	0.0000	0.6818	0.8802	0.9951	0.9306	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9087	0.9926	0.9907	0.9798
8	C	B	{100}	0.0000	0.9998	0.8917	0.6177	0.9573	0.0000	0.9564	0.9988	0.9381	0.9803	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9928	0.9566	0.8802	0.9891

(Cont.)

				{81}	{82}	{83}	{84}	{85}	{86}	{87}	{88}	{89}	{90}	{91}	{92}	{93}	{94}	{95}	{96}	{97}	{98}	{99}	{100}
Week	Treatments	Position		47.09	10.08	2.38	4.00	4.43	40.20	9.64	2.33	3.25	3.75	44.40	45.25	45.25	45.43	44.40	47.40	9.20	2.40	3.50	4.50
0	M	A	{1}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
0	M	3	{2}	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
0	M	2	{3}	0.0001	0.0326	0.0906	0.8537	0.9547	0.0000	0.0861	0.0810	0.4764	0.7750	0.0000	0.0000	0.0001	0.0001	0.0000	0.0001	0.1812	0.0875	0.6342	0.9525
0	M	1	{4}	0.0000	0.0032	0.6245	0.9697	0.9464	0.0001	0.0154	0.6070	0.9292	0.9803	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0576	0.5934	0.9626	0.7761
0	M	B	{5}	0.0001	0.0304	0.1533	0.9032	0.9686	0.0000	0.0913	0.1395	0.6005	0.8537	0.0000	0.0001	0.0001	0.0001	0.0001	0.0000	0.2145	0.1470	0.7425	0.9626
0	P	A	{6}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000
0	P	3	{7}	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
0	P	2	{8}	0.0001	0.0263	0.0941	0.8676	0.9623	0.0000	0.0684	0.0841	0.4908	0.7898	0.0000	0.0000	0.0000	0.0001	0.0000	0.0001	0.1420	0.0911	0.6501	0.9611
0	P	1	{9}	0.0000	0.0029	0.6443	0.9817	0.9765	0.0001	0.0142	0.6261	0.9419	0.9865	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0535	0.6150	0.9716	0.9054
0	P	B	{10}	0.0000	0.0109	0.3791	0.9716	0.9894	0.0000	0.0416	0.3573	0.8385	0.9611	0.0001	0.0000	0.0000	0.0000	0.0001	0.0000	0.1232	0.3619	0.9160	0.9817
0	N	A	{11}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000
0	N	3	{12}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000
0	N	2	{13}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000
0	N	1	{14}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
0	N	B	{15}	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
0	C	A	{16}	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000
0	C	3	{17}	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
0	C	2	{18}	0.0001	0.0296	0.1156	0.8849	0.9663	0.0000	0.0823	0.1041	0.5368	0.8191	0.0000	0.0001	0.0001	0.0001	0.0000	0.0001	0.1817	0.1115	0.6906	0.9635
0	C	1	{19}	0.0000	0.0001	0.9814	1.0000	0.9997	0.0000	0.0002	0.9819	0.9938	0.9722	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0012	0.9670	0.9914	1.0000
0	C	B	{20}	0.0000	0.0034	0.6209	0.9886	0.9922	0.0001	0.0160	0.6005	0.9426	0.9890	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0581	0.5947	0.9740	0.9750
2	M	A	{21}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000
2	M	3	{22}	0.0001	0.0412	0.0455	0.7425	0.9030	0.0000	0.0947	0.0401	0.3264	0.6342	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.1737	0.0442	0.4764	0.9032
2	M	2	{23}	0.0000	0.0899	0.0033	0.2691	0.4928	0.0000	0.1297	0.0027	0.0535	0.1810	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1242	0.0032	0.1026	0.5054
2	M	1	{24}	0.0000	0.0002	0.9533	0.9976	0.9485	0.0000	0.0011	0.9497	0.9976	0.9996	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0057	0.9383	0.9991	1.0000

2	M	B	{25}	0.0000	0.0098	0.3925	0.9785	0.9934	0.0000	0.0375	0.3699	0.8537	0.9682	0.0001	0.0001	0.0000	0.0000	0.0001	0.0000	0.1114	0.3759	0.9272	0.9890
2	P	A	{26}	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000
2	P	3	{27}	0.0000	0.0088	0.4058	0.9837	0.9959	0.0000	0.0334	0.3824	0.8676	0.9741	0.0001	0.0001	0.0001	0.0000	0.0001	0.0000	0.0998	0.3897	0.9369	0.9934
2	P	2	{28}	0.0000	0.0027	0.6632	0.9890	0.9899	0.0001	0.0131	0.6444	0.9525	0.9907	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0494	0.6357	0.9785	0.9612
2	P	1	{29}	0.0000	0.0000	0.9862	0.9999	0.9998	0.0000	0.0001	0.9878	0.9692	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0005	0.9692	0.8211	0.9999
2	P	B	{30}	0.0000	0.0001	0.9759	0.9722	0.9857	0.0000	0.0005	0.9747	0.9982	0.9994	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0027	0.9638	0.9990	0.9994
2	N	A	{31}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000
2	N	3	{32}	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
2	N	2	{33}	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
2	N	1	{34}	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
2	N	B	{35}	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
2	C	A	{36}	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
2	C	3	{37}	0.0000	0.0092	0.3833	0.9833	0.9962	0.0000	0.0344	0.3599	0.8572	0.9720	0.0001	0.0001	0.0001	0.0001	0.0001	0.0000	0.1005	0.3686	0.9317	0.9943
2	C	2	{38}	0.0000	0.0078	0.4564	0.9763	0.9884	0.0001	0.0320	0.4345	0.8778	0.9716	0.0001	0.0000	0.0000	0.0000	0.0001	0.0000	0.1016	0.4351	0.9383	0.9753
2	C	1	{39}	0.0000	0.0001	0.9804	0.8802	0.9953	0.0000	0.0003	0.9798	0.9981	0.9990	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0020	0.9691	0.9989	0.9997
2	C	B	{40}	0.0000	0.0025	0.6636	0.9657	0.9093	0.0001	0.0124	0.6476	0.9382	0.9815	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0483	0.6306	0.9667	0.5465
4	M	A	{41}	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
4	M	3	{42}	0.0001	0.0366	0.0386	0.7180	0.8915	0.0000	0.0791	0.0338	0.2978	0.6033	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.1348	0.0375	0.4441	0.8932
4	M	2	{43}	0.0000	0.0000	0.9989	0.9970	0.9913	0.0000	0.0000	0.9994	0.9569	0.9904	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9887	0.9865	0.9933
4	M	1	{44}	0.0000	0.0000	0.9996	0.9865	0.9580	0.0000	0.0000	0.9999	0.9844	0.9817	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9279	0.9857	0.9611
4	M	B	{45}	0.0000	0.0000	0.9993	0.9964	0.9913	0.0000	0.0000	0.9996	0.8211	0.9844	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9944	0.9692	0.9937
4	P	A	{46}	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
4	P	3	{47}	0.0000	0.0001	0.9700	0.9905	0.9314	0.0000	0.0006	0.9682	0.9981	0.9995	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0035	0.9571	0.9991	0.9988
4	P	2	{48}	0.0000	0.0000	0.9990	0.9953	0.9855	0.0000	0.0000	0.9996	0.9772	0.9895	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9838	0.9886	0.9880
4	P	1	{49}	0.0000	0.0000	0.9643	0.5993	0.3619	0.0000	0.0000	0.9537	0.8764	0.6883	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9851	0.7971	0.3425
4	P	B	{50}	0.0000	0.0001	0.9999	0.9788	0.9087	0.0000	0.0001	0.9997	0.9986	0.9877	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	1.0000	0.9957	0.9021
4	N	A	{51}	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
4	N	3	{52}	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
4	N	2	{53}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
4	N	1	{54}	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
4	N	B	{55}	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
4	C	A	{56}	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
4	C	3	{57}	0.0000	0.0689	0.0102	0.4516	0.6912	0.0000	0.1222	0.0087	0.1227	0.3353	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.1666	0.0099	0.2122	0.6992
4	C	2	{58}	0.0000	0.0000	0.9921	0.9979	0.9992	0.0000	0.0001	0.9926	0.9963	0.9639	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0005	0.9832	0.9930	0.9998

4	C	1	{59}	0.0000	0.0001	0.9973	0.9843	0.9336	0.0000	0.0001	0.9876	0.9981	0.9890	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9999	0.9955	0.9308
4	C	B	{60}	0.0000	0.0000	0.9991	0.9928	0.9770	0.0000	0.0000	0.9997	0.9846	0.9877	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9685	0.9891	0.9798
6	M	A	{61}	0.0043	0.0000	0.0000	0.0000	0.0000	0.0661	0.0000	0.0000	0.0000	0.0000	0.5183	0.3200	0.3652	0.3055	0.6200	0.0015	0.0000	0.0000	0.0000	0.0000
6	M	3	{62}	0.0000	0.0000	0.9866	1.0000	0.9989	0.0000	0.0002	0.9867	0.9976	0.9959	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0011	0.9763	0.9976	0.9998
6	M	2	{63}	0.0000	0.0001	0.9993	0.9741	0.8980	0.0000	0.0001	0.9982	0.9976	0.9837	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	1.0000	0.9937	0.8917
6	M	1	{64}	0.0000	0.0000	0.9932	0.8385	0.6357	0.0000	0.0001	0.9891	0.9716	0.8885	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9984	0.9419	0.6177
6	M	B	{65}	0.0001	0.0338	0.1282	0.8848	0.9626	0.0000	0.0974	0.1160	0.5550	0.8259	0.0000	0.0001	0.0001	0.0001	0.0000	0.0001	0.2209	0.1231	0.7037	0.9573
6	P	A	{66}	0.0116	0.0000	0.0000	0.0000	0.0000	0.0551	0.0000	0.0000	0.0000	0.0000	0.5845	0.4580	0.5186	0.4591	0.7120	0.0047	0.0000	0.0000	0.0000	0.0000
6	P	3	{67}	0.0001	0.0340	0.1147	0.8758	0.9606	0.0000	0.0955	0.1034	0.5294	0.8105	0.0000	0.0001	0.0001	0.0001	0.0000	0.0001	0.2117	0.1104	0.6818	0.9564
6	P	2	{68}	0.0000	0.0000	0.9976	0.9989	0.9979	0.0000	0.0000	0.9982	0.9399	0.9818	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9905	0.8802	0.9988
6	P	1	{69}	0.0000	0.0001	0.9882	0.9854	0.9397	0.0000	0.0001	0.9200	0.9977	0.9890	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9999	0.9951	0.9381
6	P	B	{70}	0.0000	0.0090	0.4235	0.9754	0.9897	0.0000	0.0357	0.4014	0.8633	0.9683	0.0001	0.0000	0.0000	0.0000	0.0001	0.0000	0.1101	0.4041	0.9306	0.9803
6	N	A	{71}	0.1502	0.0000	0.0000	0.0000	0.0000	0.0002	0.0000	0.0000	0.0000	0.0000	0.9753	0.9959	0.9722	0.9485	0.9553	0.1982	0.0000	0.0000	0.0000	0.0000
6	N	3	{72}	0.4076	0.0000	0.0000	0.0000	0.0000	0.0006	0.0000	0.0000	0.0000	0.0000	0.9485	0.8211	0.9722	0.9802	0.8502	0.3116	0.0000	0.0000	0.0000	0.0000
6	N	2	{73}	0.2234	0.0000	0.0000	0.0000	0.0000	0.0032	0.0000	0.0000	0.0000	0.0000	0.9955	0.7761	0.9054	0.9182	0.9279	0.1476	0.0000	0.0000	0.0000	0.0000
6	N	1	{74}	0.0237	0.0000	0.0000	0.0000	0.0000	0.0523	0.0000	0.0000	0.0000	0.0000	0.3347	0.5094	0.5934	0.5543	0.5992	0.0107	0.0000	0.0000	0.0000	0.0000
6	N	B	{75}	0.0099	0.0000	0.0000	0.0000	0.0000	0.0836	0.0000	0.0000	0.0000	0.0000	0.4144	0.3924	0.4580	0.4078	0.5845	0.0040	0.0000	0.0000	0.0000	0.0000
6	C	A	{76}	0.0004	0.0000	0.0000	0.0000	0.0000	0.1035	0.0000	0.0000	0.0000	0.0000	0.2516	0.0949	0.1108	0.0820	0.3116	0.0001	0.0000	0.0000	0.0000	0.0000
6	C	3	{77}	0.0000	0.0114	0.3275	0.9769	0.9948	0.0000	0.0405	0.3054	0.8191	0.9606	0.0001	0.0001	0.0001	0.0001	0.0001	0.0000	0.1129	0.3150	0.9087	0.9928
6	C	2	{78}	0.0000	0.0001	0.9889	0.9878	0.9554	0.0000	0.0000	0.9980	0.9946	0.9874	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.9926	0.9566
6	C	1	{79}	0.0000	0.0001	0.9969	0.9682	0.8859	0.0000	0.0001	0.9905	0.9960	0.9785	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9998	0.9907	0.8802
6	C	B	{80}	0.0000	0.0032	0.6385	0.9928	0.9964	0.0001	0.0147	0.6177	0.9521	0.9922	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0533	0.6138	0.9798	0.9891
8	M	A	{81}		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2654	0.4558	0.3424	0.2893	0.2249	0.7798	0.0000	0.0000	0.0000	0.0000
8	M	3	{82}	0.0000		0.0001	0.0000	0.0001	0.0000	0.6860	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.7037	0.0000	0.0000	0.0002
8	M	2	{83}	0.0000	0.0001		0.9904	0.9603	0.0000	0.0001	0.9630	0.9973	0.9912	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9999	0.9955	0.9607
8	M	1	{84}	0.0000	0.0000	0.9904		0.9952	0.0000	0.0002	0.9903	0.9990	0.9994	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0011	0.9830	0.9994	0.9994
8	M	B	{85}	0.0000	0.0001	0.9603	0.9952		0.0000	0.0009	0.9576	0.9977	0.9995	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0047	0.9459	0.9990	0.9977
8	P	A	{86}	0.0000	0.0000	0.0000	0.0000	0.0000		0.0000	0.0000	0.0000	0.0000	0.0028	0.0003	0.0003	0.0002	0.0036	0.0000	0.0000	0.0000	0.0000	0.0001
8	P	3	{87}	0.0000	0.6860	0.0001	0.0002	0.0009	0.0000		0.0001	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.6931	0.0000	0.0001	0.0010
8	P	2	{88}	0.0000	0.0001	0.9630	0.9903	0.9576	0.0000	0.0001		0.9981	0.9920	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9999	0.9963	0.9572
8	P	1	{89}	0.0000	0.0000	0.9973	0.9990	0.9977	0.0000	0.0000	0.9981		0.9914	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.9879	0.9722	0.9986
8	P	B	{90}	0.0000	0.0000	0.9912	0.9994	0.9995	0.0000	0.0001	0.9920	0.9914		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0005	0.9803	0.9722	0.9999
8	N	A	{91}	0.2654	0.0000	0.0000	0.0000	0.0000	0.0028	0.0000	0.0000	0.0000	0.0000		0.9396	0.9727	0.9678	1.0000	0.1681	0.0000	0.0000	0.0000	0.0000
8	N	3	{92}	0.4558	0.0000	0.0000	0.0000	0.0000	0.0003	0.0000	0.0000	0.0000	0.0000	0.9396		1.0000	0.9857	0.8686	0.3749	0.0000	0.0000	0.0000	0.0000

8	N	2	{93}	0.3424	0.0000	0.0000	0.0000	0.0000	0.0003	0.0000	0.0000	0.0000	0.0000	0.9727	1.0000		0.8717	0.9396	0.2937	0.0000	0.0000	0.0000	0.0000
8	N	1	{94}	0.2893	0.0000	0.0000	0.0000	0.0000	0.0002	0.0000	0.0000	0.0000	0.0000	0.9678	0.9857	0.8717		0.9388	0.2815	0.0000	0.0000	0.0000	0.0000
8	N	B	{95}	0.2249	0.0000	0.0000	0.0000	0.0000	0.0036	0.0000	0.0000	0.0000	0.0000	1.0000	0.8686	0.9396	0.9388		0.1430	0.0000	0.0000	0.0000	0.0000
8	C	A	{96}	0.7798	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1681	0.3749	0.2937	0.2815	0.1430		0.0000	0.0000	0.0000	0.0000
8	C	3	{97}	0.0000	0.7037	0.0000	0.0011	0.0047	0.0000	0.6931	0.0001	0.0001	0.0005	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.0000	0.0002	0.0053
8	C	2	{98}	0.0000	0.0000	0.9999	0.9830	0.9459	0.0000	0.0000	0.9999	0.9879	0.9803	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.9865	0.9482
8	C	1	{99}	0.0000	0.0000	0.9955	0.9994	0.9990	0.0000	0.0001	0.9963	0.9722	0.9722	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.9865		0.9996
8	C	B	{100}	0.0000	0.0002	0.9607	0.9994	0.9977	0.0001	0.0010	0.9572	0.9986	0.9999	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0053	0.9482	0.9996	

APPENDIX 9

Summary of 2-Way ANOVA and *Post Hoc* Test Results on Effects of Fish Feed (M = Commercially-Produced Pellet, P = Home-Made Pellet, N = Without Fish & Feed, C = Outside Cages) and Immersion Time (Wk 1, 2, 3…,8) on Biomass of Sessile and Non-Sessile Biofouling, Experiment I

Appendix 9

Summary of 2-Way ANOVA and *Post Hoc* Test Results on Effects of Fish Feed (M = Commercially-Produced Pellet, P = Home-Made Pellet, N = Without Fish & Feed, C = Outside Cages) and Immersion Time (Wk 1, 2, 3…,8) on Biomass of Sessile and Non-sessile Biofouling,

a. Sessile organisms

ANOVA results

Source of variation:	df	SS	F	p
Feed (A):	3	1.90	148.30	< 0.000001
Immersion time (B):	7	3.99	133.32	< 0.000001
A x B:	21	0.49	5.49	< 0.000001
Error:	64	0.27		

*Post hoc Test Results**

Feed (A): M(542.9) P(534.7) N(442.8) C(219.7)

Immersion time (B): wk 8(703) wk 7(657.5) wk 6(654.2) wk 5(465.5) wk 4(340) wk 3(265.6) wk 2(215.9) wk 1(178.4)

A x B: wk 1 P(198.6) N(191.1) M(181.5) C(142.5)
wk 2 P(246.9) N(232.6) M(201) C(183.2)
wk 3 M(339.7) P(282.8) N(280.4) C(159.7)
wk 4 P(411.9) M(388) N(370.3) C(190)
wk 5 M(624.1) P(526.2) N(462.8) C(249.1)
wk 6 M(841.1) P(825.7) N(623.7) C(326.2)
wk 7 M(862) P(852.6) N(690.5) C(225.1)
wk 8 P(932.7) M(906.1) N(691.4) C(281.6)

b. Non-sessile organisms

ANOVA results

Source of variation:	df	SS	F	p
Feed (A):	3	5.47	282.96	< 0.000001
Immersion time (B):	7	5.21	115.44	< 0.000001
A x B:	21	1.83	13.49	< 0.000001
Error:	64	0.41		

*Post hoc Test Results**

Feed (A): P(59.7) M(55.1) N(16.7) C(13.3)

Immersion time (B): wk 7(65.7) wk 8(56.6) wk 6(49.8) wk 5(39) wk 4(38.8) wk 3(16.3) wk 2(11.8) wk 1(11.6)

(A x B): wk 1 M(13.9) P(13.5) N(9.4) C(9.7)
wk 2 P(13.9) M(11.3) N(11.2) C(10.8)
wk 3 M(21.3) P(19.5) N(12.3) C(12.2)
wk 4 P(65.8) M(61.1) N(15) C(13.5)
wk 5 M(63.6) P(62.3) N(18.2) C(12.1)
wk 6 P(82.8) M(73.9) N(23.9) C(18.9)
wk 7 P(113.1) M(110.6) N(22.2) C(17)
wk 8 P(106.8) M(85.1) N(21.8) C(12.7)

*Based on Student Newman-Keuls (SNK) test: Mean biomass (g per panel) of each treatment (M, P, N, C) are ranked from left to right in descending order; homogeneous groups ($P > 0.05$) are underlined and joined together.

APPENDIX 10

Summary of 3 by 3 Latin Square ANOVA and Mean & Standard Deviations Results on Effects of Fish Feed (M, T, N), Net-Cage Position Along Longitudinal (D, I, U) and Cross River (R, I, B), on Biomass of Sessile and Non-Sessile Biofouling Each Weeks (Wk 1, 2, 3….8) (a), and 2-Way ANOVA and *Post Hoc* Test Results on Effects of Fish Feed (M, T, N) and Immersion Time (Wk 1, 2, 3…,8) on Biomass of Biofouling After Removing the "Position" Factor (b), Experiment II

Summary of 3 by 3 Latin Square ANOVA and Mean & Standard Deviations Results on Effects of Fish Feed (M, T, N), Net-Cage Position Along Longitudinal (D, I, U) and Cross River (R, I, B) on Biomass of Sessile and Non-Sessile Biofouling Each Weeks (Wk 1, 2, 3….8) (a), and 2-Way ANOVA and *Post Hoc* Test Results on Effects of Fish Feed (M, T, N) and Immersion Time (Wk 1, 2, 3…,8) on Biomass of Biofouling After Removing the "Position" Factor (b), Experiment II

a) i. Sessile biofouling

Week 1

ANOVA results(3 by 3 Latin Square)

Effect	SS	df	MS	F	p
Feed	3938.3620	2	1969.1810	43.9375	0.0223
Longitudinal	1041.5560	2	520.7778	11.6199	0.0792
Cross river	416.5489	2	208.2744	4.6471	0.1771
Residual	89.6356	2	44.8178		

Mean & Standard Deviations

	Level	Means	Paramet.	Std.Dev.
Feed	M	219.0667	13.1111	18.8850
	T	222.3667	16.4111	19.1892
	N	176.4333	-29.5222	7.0002
Longitudinal	D	193.0667	-12.8889	13.5736
	I	205.4000	-0.5556	32.2068
	U	219.4000	13.4444	31.6346
Cross river	R	215.5000	9.5444	33.2811
	I	200.1333	-5.8222	17.0530
	B	202.2333	-3.7222	33.7097

Week 2

ANOVA results (3 by 3 Latin Square)

Effect	SS	df	MS	F	p
Feed	1529.7490	2	764.8745	0.1136	0.8980
Longitudinal	21398.4200	2	10699.2100	1.5895	0.3862
Crossriver	13566.8000	2	6783.4010	1.0078	0.4981
Residual	13462.1400	2	6731.0680		

Mean & Standard Deviations

	Level	Means	Paramet.	Std.Dev.
Feed	M	284.1000	-10.9444	79.9182
	T	287.6667	-7.3778	119.6405
	N	313.3667	18.3222	59.2698
Longitudinal	D	242.2000	-52.8444	32.7822
	I	283.1000	-11.9444	87.1411
	U	359.8333	64.7889	74.9073
Cross river	R	290.6667	-4.3778	79.7966
	I	249.8333	-45.2111	29.0097
	B	344.6333	49.5889	104.8145

Week 3

ANOVA results (3 by 3 Latin Square)

Effect	SS	df	MS	F	p

Feed	14244.7800	2	7122.3880	1.2277	0.4489
Longitudinal	15323.4000	2	7661.7010	1.3207	0.4309
Cross river	7557.0420	2	3778.5210	0.6513	0.6056
Residual	11602.7800	2	5801.3910		

Mean & Standard Deviations

	Level	Means	Paramet.	Std.Dev.
Feed	M	250.2333	-10.9111	19.5595
	T	218.8000	-42.3444	37.7718
	N	314.4000	53.2556	124.2269
Longitudinal	D	239.2667	-21.8778	60.2963
	I	225.2333	-35.9111	37.7022
	U	318.9333	57.7889	107.9129
Cross river	R	256.7333	-4.4111	45.9156
	I	298.6333	37.4889	132.1439
	B	228.0667	-33.0778	31.8626

Week 4

ANOVA results (3 by 3 Latin Square)

Effect	SS	df	MS	F	p
Feed	14781.3000	2	7390.6480	3.2773	0.2338
Longitudinal	1631.5290	2	815.7645	0.3617	0.7344
Cross river	1250.4620	2	625.2311	0.2772	0.7829
Residual	4510.2420	2	2255.1210		

Mean & Standard Deviations

	Level	Means	Paramet.	Std.Dev.
Feed	M	305.8000	34.0889	31.4387
	T	294.5667	22.8556	38.7513
	N	214.7667	-56.9444	34.7284
Longitudinal	D	261.4667	-10.2444	35.3023
	I	262.9333	-8.7778	82.6459
	U	290.7333	19.0222	46.8445
Cross river	R	259.4000	-12.3111	34.0912
	I	287.6000	15.8889	49.4197
	B	268.1333	-3.5778	82.8071

Week 5

ANOVA results (3 by 3 Latin Square)

Effect	SS	df	MS	F	p
Feed	39354.6200	2	19677.3100	1.5765	0.3881
Longitudinal	2364.1490	2	1182.0740	0.0947	0.9135
Cross river	7441.7090	2	3720.8540	0.2981	0.7704
Residual	24962.9100	2	12481.4500		

Mean & Standard Deviations

	Level	Means	Paramet.	Std.Dev.
Feed	M	443.9000	3.4444	115.9066
	T	519.6667	79.2111	48.8817
	N	357.8000	-82.6556	39.5048
Longitudinal	D	427.8000	-12.6556	126.2120
	I	430.2333	-10.2222	57.8018
	U	463.3333	22.8778	128.8763
Cross river	R	472.1333	31.6778	79.4257
	I	446.7000	6.2444	126.2655
	B	402.5333	-37.9222	105.3063

Week 6

ANOVA results (3 by 3 Latin Square)

Effect	SS	df	MS	F	p
Feed	134342.7000	2	67171.3400	14.3079	0.0653
Longitudinal	2579.6820	2	1289.8410	0.2747	0.7845
Cross river	39586.9600	2	19793.4800	4.2161	0.1917

Residual	9389.4160	2	4694.7080	

Mean & Standard Deviations

	Level	Means	Paramet.	Std.Dev.
Feed	M	707.9333	71.0444	100.6571
	T	737.7667	100.8778	121.3728
	N	464.9667	-171.9222	30.2459
Longitudinal	D	615.6667	-21.2222	165.2202
	I	637.9000	1.0111	165.9233
	U	657.1000	20.2111	191.9147
Cross river	R	673.5000	36.6111	189.5552
	I	543.8000	-93.0889	91.9001
	B	693.3666	56.4778	169.6439

Week 7

ANOVA results (3 by 3 Latin Square)

Effect	SS	df	MS	F	p
Feed	138097.0000	2	69048.5100	3.5375	0.2204
Longitudinal	69688.9900	2	34844.5000	1.7852	0.3590
Cross river	27799.9500	2	13899.9700	0.7121	0.5841
Residual	39037.6000	2	19518.8000		

Mean & Standard Deviations

	Level	Means	Paramet.	Std.Dev.
Feed	M	791.1334	123.4067	21.2890
	T	713.7000	45.9733	252.0166
	N	498.3467	-169.3800	65.5567
Longitudinal	D	559.3333	-108.3933	205.4175
	I	774.8666	107.1400	201.0993
	U	668.9800	1.2533	140.8190
Cross river	R	717.6000	49.8733	267.4570
	I	590.1800	-77.5467	191.8772
	B	695.4000	27.6733	122.7261

Week 8

ANOVA results (3 by 3 Latin Square)

Effect	SS	df	MS	F	p
Feed	133174.2000	2	66587.1100	5.1055	0.1638
Longitudinal	184.3400	2	92.1700	0.0071	0.9930
Cross river	26659.9800	2	13329.9900	1.0221	0.4945
Residual	26084.6600	2	13042.3300		

Mean & Standard Deviations

	Level	Means	Paramet.	Std.Dev.
Feed	M	817.7000	105.4667	100.1899
	T	777.2000	64.9667	122.2720
	N	541.8000	-170.4333	38.4193
Longitudinal	D	718.2667	6.0333	186.7302
	I	707.3666	-4.8667	178.0647
	U	711.0667	-1.1667	162.4322
Cross river	R	742.3333	30.1000	142.5351
	I	635.8333	-76.4000	109.0584
	B	758.5333	46.3000	217.9716

a) ii. Non-sessile biofouling

Week 1

ANOVA results (3 by 3 Latin Square)

Effect	SS	df	MS	F	p
Feed	3.2822	2	1.6411	4.3061	0.1885
Longitudinal	2.5356	2	1.2678	3.3265	0.2311

Cross river	0.5422	2	0.2711	0.7114	0.5843
Residual	0.7622	2	0.3811		

Mean & Standard Deviations

	Level	Means	Paramet.	Std.Dev.
Feed	M	9.9667	0.8222	1.2662
	T	8.9333	-0.2111	0.5132
	N	8.5333	-0.6111	0.2309
Longitudinal	D	9.1333	-0.0111	0.9452
	I	9.8000	0.6556	1.1790
	U	8.5000	-0.6444	0.1000
Cross river	R	8.8333	-0.3111	0.5859
	I	9.4333	0.2889	1.4572
	B	9.1667	0.0222	0.9074

Week 2

ANOVA results (3 by 3 Latin Square)

Effect	SS	df	MS	F	p
Feed	167.0556	2	83.5278	10.6164	0.0861
Longitudinal	35.6822	2	17.8411	2.2676	0.3060
Cross river	19.4956	2	9.7478	1.2389	0.4466
Residual	15.7356	2	7.8678		

Mean & Standard Deviations

	Level	Means	Paramet.	Std.Dev.
Feed	M	20.0667	5.5556	5.5788
	T	13.9000	-0.6111	1.7521
	N	9.5667	-4.9444	1.1240
Longitudinal	D	16.3000	1.7889	5.8232
	I	15.5000	0.9889	7.8886
	U	11.7333	-2.7778	2.2368
Cross river	R	12.7667	-1.7444	1.7039
	I	16.3667	1.8556	7.4225
	B	14.4000	-0.1111	7.1582

Week 3

ANOVA results (3 by 3 Latin Square)

Effect	SS	df	MS	F	p
Feed	3154.2160	2	1577.1080	3.9213	0.2032
Longitudinal	837.8956	2	418.9478	1.0417	0.4898
Cross river	498.7622	2	249.3811	0.6201	0.6173
Residual	804.3822	2	402.1911		

Mean & Standard Deviations

	Level	Means	Paramet.	Std.Dev.
Feed	M	55.2667	18.9444	1.1930
	T	42.8667	6.5444	32.4446
	N	10.8333	-25.4889	4.0550
Longitudinal	D	24.4333	-11.8889	25.5348
	I	36.4667	0.1444	26.0608
	U	48.0667	11.7444	29.9582
Cross river	R	37.6000	1.2778	23.8504
	I	26.6333	-9.6889	25.7065
	B	44.7333	8.4111	34.1846

Week 4

ANOVA results (3 by 3 Latin Square)

Effect	SS	df	MS	F	p
Feed	4694.6460	2	2347.3230	7.1946	0.1220
Longitudinal	503.1800	2	251.5900	0.7711	0.5646
Cross river	99.7067	2	49.8533	0.1528	0.8675
Residual	652.5267	2	326.2633		

Mean & Standard Deviations

	Level	Means	Paramet.	Std.Dev.
Feed	M	61.2667	19.6000	11.2216
	T	54.1000	12.4333	22.3938
	N	9.6333	-32.0333	0.5508
Longitudinal	D	32.7333	-8.9333	20.5554
	I	41.2333	-0.4333	30.3266
	U	51.0333	9.3667	37.1647
Cross river	R	38.8000	-2.8667	27.4585
	I	39.8667	-1.8000	30.8503
	B	46.3333	4.6667	34.9208

Week 5

ANOVA results (3 by 3 Latin Square)

Effect	SS	df	MS	F	p
Feed	10205.8800	2	5102.9380	44.1885	0.0221
Longitudinal	152.1356	2	76.0678	0.6587	0.6029
Cross river	187.5756	2	93.7878	0.8121	0.5518
Residual	230.9622	2	115.4811		

Mean & Standard Deviations

	Level	Means	Paramet.	Std.Dev.
Feed	M	96.6333	28.0445	7.0117
	T	87.9000	19.3111	8.2529
	N	21.2333	-47.3556	12.9639
Longitudinal	D	73.9333	5.3444	34.7773
	I	63.9333	-4.6556	43.3545
	U	67.9000	-0.6889	47.1501
Cross river	R	71.7333	3.1444	30.9944
	I	62.1333	-6.4556	42.4818
	B	71.9000	3.3111	50.2905

Week 6

ANOVA results (3 by 3 Latin Square)

Effect	SS	df	MS	F	p
Feed	5645.5620	2	2822.7810	427.6221	0.0023
Longitudinal	273.1089	2	136.5544	20.6866	0.0461
Cross river	844.5956	2	422.2978	63.9737	0.0154
Residual	13.2022	2	6.6011		

Mean & Standard Deviations

	Level	Means	Paramet.	Std.Dev.
Feed	M	78.5000	16.4111	11.0729
	T	81.0667	18.9778	11.7985
	N	26.7000	-35.3889	17.4253
Longitudinal	D	69.7333	7.6444	20.1431
	I	59.5667	-2.5222	36.6596
	U	56.9667	-5.1222	38.7558
Cross river	R	75.7333	13.6444	25.3843
	I	54.2000	-7.8889	35.9418
	B	56.3333	-5.7556	32.0899

Week 7

ANOVA results (3 by 3 Latin Square)

Effect	SS	df	MS	F	p
Feed	6494.2420	2	3247.1210	24.3922	0.0394
Longitudinal	64.1356	2	32.0678	0.2409	0.8059
Cross river	463.6956	2	231.8478	1.7416	0.3647
Residual	266.2422	2	133.1211		

Mean & Standard Deviations

	Level	Means	Paramet.	Std.Dev.
Feed	M	74.0667	11.0889	10.5121
	T	88.9000	25.9222	14.8314

	N	25.9667	-37.0111	8.1586
Longitudinal	D	64.2333	1.2556	27.1353
	I	65.4333	2.4556	37.6819
	U	59.2667	-3.7111	38.1555
Cross river	R	67.7333	4.7556	33.8500
	I	52.8333	-10.1444	30.5886
	B	68.3667	5.3889	36.4805

Week 8

ANOVA results (3 by 3 Latin Square)

Effect	SS	df	MS	F	p
Feed	16887.4800	2	8443.7410	14.2284	0.0657
Longitudinal	3152.7360	2	1576.3680	2.6563	0.2735
Cross river	419.3089	2	209.6544	0.3533	0.7389
Residual	1186.8890	2	593.4445		

Mean & Standard Deviations

	Level	Means	Paramet.	Std.Dev.
Feed	M	114.5000	32.7222	40.8685
	T	110.2667	28.4889	25.3277
	N	20.5667	-61.2111	8.2306
Longitudinal	D	97.4333	15.6556	70.5351
	I	92.4333	10.6556	54.3785
	U	55.4667	-26.3111	36.2576
Cross river	R	72.2333	-9.5444	54.8573
	I	85.3000	3.5222	61.7565
	B	87.8000	6.0222	61.5659

b) i. Sessile biofouling

ANOVA results

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Week	7	364261.2188	48	8361.4775	43.5642	0.0000
Feed	2	128721.4219	48	8361.4775	15.3946	0.0000
Week x Feed	14	24591.4180	48	8361.4775	2.9410	0.0027

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}
Week	Feed		219.07	222.37	176.43	284.10	287.67	313.37	250.23	218.80	314.40	305.80	294.57	214.77
1	M	{1}		0.9650	0.9403	0.8198	0.8883	0.9078	0.9087	0.9973	0.9333	0.9049	0.9120	0.9983
1	T	{2}	0.9650		0.9720	0.6884	0.8180	0.8834	0.7107	0.9988	0.9180	0.8718	0.8686	0.9997
1	N	{3}	0.9403	0.9720		0.7764	0.8090	0.7544	0.9195	0.8381	0.7836	0.7716	0.8095	0.6101
2	M	{4}	0.8198	0.6884	0.7764		0.9622	0.9949	0.6523	0.9049	0.9986	0.9914	0.9893	0.9372
2	T	{5}	0.8883	0.8180	0.8090	0.9622		0.9859	0.8711	0.9389	0.9964	0.9681	0.9269	0.9568
2	N	{6}	0.9078	0.8834	0.7544	0.9949	0.9859		0.9573	0.9361	0.9891	0.9198	0.9658	0.9438
3	M	{7}	0.9087	0.7107	0.9195	0.6523	0.8711	0.9573		0.9747	0.9768	0.9449	0.9336	0.9893
3	T	{8}	0.9973	0.9988	0.8381	0.9049	0.9389	0.9361	0.9747		0.9534	0.9378	0.9482	0.9572
3	N	{9}	0.9333	0.9180	0.7836	0.9986	0.9964	0.9891	0.9768	0.9534		0.9928	0.9934	0.9577
4	M	{10}	0.9049	0.8718	0.7716	0.9914	0.9681	0.9198	0.9449	0.9378	0.9928		0.8811	0.9482
4	T	{11}	0.9120	0.8686	0.8095	0.9893	0.9269	0.9658	0.9336	0.9482	0.9934	0.8811		0.9603
4	N	{12}	0.9983	0.9997	0.6101	0.9372	0.9568	0.9438	0.9893	0.9572	0.9577	0.9482	0.9603	
5	M	{13}	0.1370	0.1330	0.0489	0.4596	0.4342	0.4152	0.2487	0.1522	0.3176	0.4449	0.4280	0.1502
5	T	{14}	0.0145	0.0146	0.0037	0.0989	0.0969	0.1291	0.0362	0.0161	0.1081	0.1237	0.1036	0.0153
5	N	{15}	0.6958	0.6730	0.4425	0.9545	0.9342	0.8235	0.8337	0.7378	0.5639	0.8980	0.9145	0.7439
6	M	{16}	0.0002	0.0001	0.0002	0.0002	0.0002	0.0002	0.0001	0.0002	0.0002	0.0002	0.0002	0.0002
6	T	{17}	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0001	0.0001	0.0002
6	N	{18}	0.0805	0.0792	0.0255	0.3360	0.3209	0.3409	0.1617	0.0893	0.2739	0.3514	0.3243	0.0871

7	M	{19}	0.0002	0.0002	0.0002	0.0002	0.0002	0.0001	0.0002	0.0002	0.0001	0.0001	0.0001	0.0002
7	T	{20}	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002
7	N	{21}	0.0287	0.0286	0.0078	0.1640	0.1583	0.1906	0.0661	0.0319	0.1557	0.1890	0.1650	0.0306
8	M	{22}	0.0002	0.0002	0.0002	0.0002	0.0002	0.0001	0.0002	0.0002	0.0001	0.0001	0.0002	0.0002
8	T	{23}	0.0002	0.0002	0.0002	0.0002	0.0001	0.0001	0.0002	0.0002	0.0002	0.0001	0.0001	0.0002
8	N	{24}	0.1284	0.1230	0.0471	0.4222	0.3898	0.3248	0.2282	0.1445	0.2134	0.3735	0.3732	0.1439

(Cont.)

			{13}	{14}	{15}	{16}	{17}	{18}	{19}	{20}	{21}	{22}	{23}	{24}
Week	Feed		443.90	519.67	357.80	707.93	737.77	464.97	791.13	713.70	498.35	817.70	777.20	441.80
1	M	{1}	0.1370	0.0145	0.6958	0.0002	0.0002	0.0805	0.0002	0.0002	0.0287	0.0002	0.0002	0.1284
1	T	{2}	0.1330	0.0146	0.6730	0.0001	0.0002	0.0792	0.0002	0.0002	0.0286	0.0002	0.0002	0.1230
1	N	{3}	0.0489	0.0037	0.4425	0.0002	0.0002	0.0255	0.0002	0.0002	0.0078	0.0002	0.0002	0.0471
2	M	{4}	0.4596	0.0989	0.9545	0.0002	0.0002	0.3360	0.0002	0.0002	0.1640	0.0002	0.0002	0.4222
2	T	{5}	0.4342	0.0969	0.9342	0.0002	0.0002	0.3209	0.0002	0.0002	0.1583	0.0002	0.0001	0.3898
2	N	{6}	0.4152	0.1291	0.8235	0.0002	0.0002	0.3409	0.0001	0.0002	0.1906	0.0001	0.0001	0.3248
3	M	{7}	0.2487	0.0362	0.8337	0.0001	0.0002	0.1617	0.0002	0.0002	0.0661	0.0002	0.0002	0.2282
3	T	{8}	0.1522	0.0161	0.7378	0.0002	0.0002	0.0893	0.0002	0.0002	0.0319	0.0002	0.0002	0.1445
3	N	{9}	0.3176	0.1081	0.5639	0.0002	0.0002	0.2739	0.0001	0.0002	0.1557	0.0001	0.0002	0.2134
4	M	{10}	0.4449	0.1237	0.8980	0.0002	0.0001	0.3514	0.0001	0.0002	0.1890	0.0001	0.0001	0.3735
4	T	{11}	0.4280	0.1036	0.9145	0.0002	0.0001	0.3243	0.0001	0.0002	0.1650	0.0002	0.0001	0.3732
4	N	{12}	0.1502	0.0153	0.7439	0.0002	0.0002	0.0871	0.0002	0.0002	0.0306	0.0002	0.0002	0.1439
5	M	{13}		0.7417	0.4867	0.0079	0.0048	0.7792	0.0009	0.0090	0.7475	0.0004	0.0013	0.9778
5	T	{14}	0.7417		0.2716	0.0152	0.0264	0.7455	0.0085	0.0327	0.7766	0.0040	0.0100	0.8341
5	N	{15}	0.4867	0.2716		0.0006	0.0003	0.4841	0.0002	0.0006	0.3406	0.0001	0.0002	0.2663
6	M	{16}	0.0079	0.0152	0.0006		0.9160	0.0110	0.7982	0.9388	0.0195	0.6844	0.7902	0.0103
6	T	{17}	0.0048	0.0264	0.0003	0.9160		0.0080	0.7561	0.7487	0.0194	0.7090	0.5999	0.0056
6	N	{18}	0.7792	0.7455	0.4841	0.0110	0.0080		0.0017	0.0139	0.6570	0.0008	0.0023	0.9485
7	M	{19}	0.0009	0.0085	0.0002	0.7982	0.7561	0.0017		0.7287	0.0050	0.7237	0.8529	0.0010
7	T	{20}	0.0090	0.0327	0.0006	0.9388	0.7487	0.0139	0.7287		0.0290	0.6351	0.6738	0.0111
7	N	{21}	0.7475	0.7766	0.3406	0.0195	0.0194	0.6570	0.0050	0.0290		0.0022	0.0064	0.8731
8	M	{22}	0.0004	0.0040	0.0001	0.6844	0.7090	0.0008	0.7237	0.6351	0.0022		0.8509	0.0005
8	T	{23}	0.0013	0.0100	0.0002	0.7902	0.5999	0.0023	0.8529	0.6738	0.0064	0.8509		0.0015
8	N	{24}	0.9778	0.8341	0.2663	0.0103	0.0056	0.9485	0.0010	0.0111	0.8731	0.0005	0.0015	

b) ii. Non-sessile biofouling

ANOVA results

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Week	7	6157.8296	48	223.4540	27.5575	0.0000
Feed	2	16797.3613	48	223.4540	75.1714	0.0000
Week x Feed	14	975.5457	48	223.4540	4.3658	0.0001

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}
Week	Feed		9.97	8.93	8.53	20.07	13.90	9.57	55.27	42.87	10.83	61.27	54.10	9.63
1	M	{1}		0.9998	1.0000	0.8412	0.9445	0.9995	0.0206	0.1768	0.9438	0.0059	0.0227	0.9784
1	T	{2}	0.9998		0.9741	0.9689	0.9985	0.9589	0.0246	0.2210	0.9999	0.0068	0.0283	0.9983
1	N	{3}	1.0000	0.9741		0.9798	0.9994	0.9961	0.0253	0.2301	1.0000	0.0069	0.0293	0.9998
2	M	{4}	0.8412	0.9689	0.9798		0.6158	0.9541	0.0988	0.4338	0.7313	0.0360	0.0991	0.9118
2	T	{5}	0.9445	0.9985	0.9994	0.6158		0.9965	0.0348	0.2324	0.8028	0.0108	0.0364	0.9852
2	N	{6}	0.9995	0.9589	0.9961	0.9541	0.9965		0.0251	0.2181	0.9996	0.0070	0.0284	0.9958
3	M	{7}	0.0206	0.0246	0.0253	0.0988	0.0348	0.0251		0.5706	0.0212	0.6254	0.9244	0.0222
3	T	{8}	0.1768	0.2210	0.2301	0.4338	0.2324	0.2181	0.5706		0.1725	0.4411	0.3621	0.1939
3	N	{9}	0.9438	0.9999	1.0000	0.7313	0.8028	0.9996	0.0212	0.1725		0.0062	0.0229	0.9948
4	M	{10}	0.0059	0.0068	0.0069	0.0360	0.0108	0.0070	0.6254	0.4411	0.0062		0.8277	0.0062

4	T	{11}	0.0227	0.0283	0.0293	0.0991	0.0364	0.0284	0.9244	0.3621	0.0229	0.8277		0.0249
4	N	{12}	0.9784	0.9983	0.9998	0.9118	0.9852	0.9958	0.0222	0.1939	0.9948	0.0062	0.0249	
5	M	{13}	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0283	0.0023	0.0002	0.0771	0.0270	0.0002
5	T	{14}	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0997	0.0124	0.0002	0.2041	0.1034	0.0002
5	N	{15}	0.9387	0.9834	0.9880	0.9951	0.9314	0.9785	0.0769	0.2990	0.9127	0.0298	0.0700	0.9620
6	M	{16}	0.0002	0.0002	0.0002	0.0009	0.0003	0.0002	0.2403	0.0563	0.0002	0.3430	0.2821	0.0002
6	T	{17}	0.0002	0.0002	0.0002	0.0006	0.0002	0.0002	0.2312	0.0439	0.0002	0.3762	0.2526	0.0002
6	N	{18}	0.8657	0.9270	0.9367	0.9823	0.8988	0.9201	0.1032	0.1917	0.8486	0.0504	0.0739	0.8930
7	M	{19}	0.0003	0.0004	0.0004	0.0022	0.0006	0.0004	0.2816	0.0953	0.0004	0.2997	0.3688	0.0004
7	T	{20}	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.1066	0.0122	0.0002	0.2290	0.1063	0.0002
7	N	{21}	0.8436	0.9227	0.9349	0.9625	0.8592	0.9126	0.1328	0.3569	0.8151	0.0602	0.1112	0.8795
8	M	{22}	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0006	0.0001	0.0002	0.0022	0.0006	0.0002
8	T	{23}	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0014	0.0002	0.0002	0.0048	0.0013	0.0002
8	N	{24}	0.9070	0.9788	0.9855	0.9676	0.8490	0.9707	0.0875	0.3705	0.8553	0.0327	0.0844	0.9457

(Cont.)

			{13}	{14}	{15}	{16}	{17}	{18}	{19}	{20}	{21}	{22}	{23}	{24}
Week	Feed		96.63	87.90	21.23	78.50	81.07	26.70	74.07	88.90	25.97	114.50	110.27	20.57
1	M	{1}	0.0002	0.0002	0.9387	0.0002	0.0002	0.8657	0.0003	0.0002	0.8436	0.0002	0.0002	0.9070
1	T	{2}	0.0002	0.0002	0.9834	0.0002	0.0002	0.9270	0.0004	0.0002	0.9227	0.0002	0.0002	0.9788
1	N	{3}	0.0002	0.0002	0.9880	0.0002	0.0002	0.9367	0.0004	0.0002	0.9349	0.0002	0.0002	0.9855
2	M	{4}	0.0002	0.0002	0.9951	0.0009	0.0006	0.9823	0.0022	0.0002	0.9625	0.0002	0.0002	0.9676
2	T	{5}	0.0002	0.0002	0.9314	0.0003	0.0002	0.8988	0.0006	0.0002	0.8592	0.0002	0.0002	0.8490
2	N	{6}	0.0002	0.0002	0.9785	0.0002	0.0002	0.9201	0.0004	0.0002	0.9126	0.0002	0.0002	0.9707
3	M	{7}	0.0283	0.0997	0.0769	0.2403	0.2312	0.1032	0.2816	0.1066	0.1328	0.0006	0.0014	0.0875
3	T	{8}	0.0023	0.0124	0.2990	0.0563	0.0439	0.1917	0.0953	0.0122	0.3569	0.0001	0.0002	0.3705
3	N	{9}	0.0002	0.0002	0.9127	0.0002	0.0002	0.8486	0.0004	0.0002	0.8151	0.0002	0.0002	0.8553
4	M	{10}	0.0771	0.2041	0.0298	0.3430	0.3762	0.0504	0.2997	0.2290	0.0602	0.0022	0.0048	0.0327
4	T	{11}	0.0270	0.1034	0.0700	0.2821	0.2526	0.0739	0.3688	0.1063	0.1112	0.0006	0.0013	0.0844
4	N	{12}	0.0002	0.0002	0.9620	0.0002	0.0002	0.8930	0.0004	0.0002	0.8795	0.0002	0.0002	0.9457
5	M	{13}		0.7556	0.0001	0.5766	0.5829	0.0002	0.4454	0.5295	0.0001	0.3173	0.2697	0.0001
5	T	{14}	0.7556		0.0002	0.7230	0.5783	0.0004	0.6710	0.9351	0.0004	0.2051	0.2711	0.0002
5	N	{15}	0.0001	0.0002		0.0008	0.0006	0.8956	0.0019	0.0002	0.7000	0.0001	0.0001	0.9568
6	M	{16}	0.5766	0.7230	0.0008		0.8344	0.0019	0.7182	0.8293	0.0021	0.0682	0.1164	0.0009
6	T	{17}	0.5829	0.5783	0.0006	0.8344		0.0013	0.8349	0.7979	0.0014	0.0860	0.1351	0.0006
6	N	{18}	0.0002	0.0004	0.8956	0.0019	0.0013		0.0042	0.0004	0.9524	0.0001	0.0001	0.9582
7	M	{19}	0.4454	0.6710	0.0019	0.7182	0.8349	0.0042		0.7426	0.0047	0.0346	0.0656	0.0020
7	T	{20}	0.5295	0.9351	0.0002	0.8293	0.7979	0.0004	0.7426		0.0004	0.1687	0.1973	0.0002
7	N	{21}	0.0001	0.0004	0.7000	0.0021	0.0014	0.9524	0.0047	0.0004		0.0001	0.0001	0.8980
8	M	{22}	0.3173	0.2051	0.0001	0.0682	0.0860	0.0001	0.0346	0.1687	0.0001		0.7303	0.0002
8	T	{23}	0.2697	0.2711	0.0001	0.1164	0.1351	0.0001	0.0656	0.1973	0.0001	0.7303		0.0001
8	N	{24}	0.0001	0.0002	0.9568	0.0009	0.0006	0.9582	0.0020	0.0002	0.8980	0.0002	0.0001	

APPENDIX 11

Summary of 2-Way Repeated Measures ANOVA and *Post Hoc* Test Results on the Effects of Salinity (10, 15, 20, 25, 30 ppt) and Immersion Time (Wk 0, 1, 2, 3) on Percentage Cover of Sessile Biofouling Species

Appendix 11

Summary of 2-Way Repeated Measures ANOVA and *Post Hoc* Test Results on the Effects of Salinity (10, 15, 20, 25, 30 ppt) and Immersion Time (Wk 0, 1, 2, 3) on Percentage Cover of Sessile Biofouling Species

a. *Plumularia* sp.

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	169717.4793	1	169717.4793	212.1874	0.0000
Salinity	20024.8833	4	5006.2208	6.2590	0.0005
Error	31993.8792	40	799.8470		
Week	1313.9719	3	437.9906	6.6200	0.0004
Week x Salinity	3142.7579	12	261.8965	3.9584	0.0000
Error	7939.4209	120	66.1618		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}	{17}	{18}	{19}	{20}
Salinity	Week		58.27	59.79	59.51	62.23	47.71	44.19	41.09	41.89	52.38	51.74	52.05	55.03	49.12	39.10	35.89	51.59	51.58	51.30	33.33	36.35
10	0	{1}		0.9179	0.7479	0.9686	0.9999	0.9985	0.9815	0.9965	1.0000	0.9902	0.9987	0.9978	0.6443	0.2985	0.5163	0.0050	0.5691	0.0047	0.7150	0.5059
10	1	{2}	0.9179		0.9427	0.9880	0.9865	1.0000	0.8617	0.9921	0.9999	0.9883	0.9981	0.9986	0.0158	0.9692	0.5824	0.0078	0.0071	0.5828	0.7647	0.5787
10	2	{3}	0.7479	0.9427		0.9918	0.9895	0.9994	0.9924	0.9977	0.9999	0.9906	1.0000	0.9991	0.0158	0.3710	0.9887	0.0077	0.0070	0.0071	0.9957	0.5887
10	3	{4}	0.9686	0.9880	0.9918		0.9474	0.9627	0.9999	0.9997	0.9837	0.9999	0.9806	0.9968	0.0203	0.3787	0.5314	0.5955	0.0106	0.0101	0.6718	0.9617
15	0	{5}	0.9999	0.9865	0.9895	0.9474		0.6305	0.7296	0.7348	0.9782	0.9925	0.9732	0.7205	0.5998	0.5473	0.5190	0.0512	0.6299	0.0367	0.4534	0.6542
15	1	{6}	0.9985	1.0000	0.9994	0.9627	0.6305		0.9838	0.9751	0.8094	1.0000	0.9917	0.9108	0.0287	0.9187	0.5218	0.0197	0.0167	0.5305	0.6123	0.5809
15	2	{7}	0.9815	0.8617	0.9924	0.9999	0.7296	0.9838		0.9761	1.0000	0.9308	0.9999	0.9995	0.0235	0.4455	0.9880	0.0122	0.0111	0.0111	0.9950	0.6595
15	3	{8}	0.9965	0.9921	0.9977	0.9997	0.7348	0.9751	0.9761		0.9999	0.9836	0.9837	0.9999	0.0246	0.4402	0.6306	0.6450	0.0121	0.0119	0.7854	0.9836
20	0	{9}	1.0000	0.9999	0.9999	0.9837	0.9782	0.8094	1.0000	0.9999		0.9998	0.9959	0.7700	0.5491	0.3406	0.4654	0.0112	0.5248	0.0091	0.5859	0.5046
20	1	{10}	0.9902	0.9883	0.9906	0.9999	0.9925	1.0000	0.9308	0.9836	0.9998		0.9965	0.9780	0.0266	0.9741	0.6694	0.0143	0.0129	0.6199	0.8241	0.6753
20	2	{11}	0.9987	0.9981	1.0000	0.9806	0.9732	0.9917	0.9999	0.9837	0.9959	0.9965		0.9368	0.0224	0.4102	0.9655	0.0129	0.0113	0.0110	0.9783	0.6032

20	3	{12}	0.9978	0.9986	0.9991	0.9968	0.7205	0.9108	0.9995	0.9999	0.7700	0.9780	0.9368		0.0307		0.4691	0.6091	0.0192	0.0173	0.5090	0.9135
25	0	{13}	0.6443	0.0158	0.0158	0.0203	0.5998	0.0287	0.0235	0.0246	0.5491	0.0266	0.0224	0.0307		0.0103	0.0043	0.9171	0.9815	0.7703	0.2252	0.2081
25	1	{14}	0.2985	0.9692	0.3710	0.3787	0.5473	0.9187	0.4455	0.4402	0.3406	0.9741	0.4102	0.4182	0.0103		0.6795	0.0126	0.3458	0.6343	0.8653	0.7126
25	2	{15}	0.5163	0.5824	0.9887	0.5314	0.5190	0.5218	0.9880	0.6306	0.4654	0.6694	0.9655	0.4691	0.0043	0.6795		0.0016	0.2976	0.2464	0.8490	0.9510
25	3	{16}	0.0050	0.0078	0.0077	0.5955	0.0512	0.0197	0.0122	0.6450	0.0112	0.0143	0.0129	0.6091	0.9171	0.0126	0.0016		0.9988	0.9993	0.2360	0.8602
30	0	{17}	0.5691	0.0071	0.0070	0.0106	0.6299	0.0167	0.0111	0.0121	0.5248	0.0129	0.0113	0.0192	0.9815	0.3458	0.2976	0.9988		0.9428	0.0002	0.0013
30	1	{18}	0.0047	0.5828	0.0071	0.0101	0.0367	0.5305	0.0111	0.0119	0.0091	0.6199	0.0110	0.0173	0.7703	0.6343	0.2464	0.9993	0.9428		0.0002	0.0010
30	2	{19}	0.7150	0.7647	0.9957	0.6718	0.4534	0.6123	0.9950	0.7854	0.5859	0.8241	0.9783	0.5090	0.2252	0.8653	0.8490	0.2360	0.0002	0.0002		0.7119
30	3	{20}	0.5059	0.5787	0.5887	0.9617	0.6542	0.5809	0.6595	0.9836	0.5046	0.6753	0.6032	0.9135	0.2081	0.7126	0.9510	0.8602	0.0013	0.0010	0.7119	

b. Anthozoans (unidentified sea anemone)

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	6332.3912	1	6332.3912	188.1939	0.0000
Salinity	1263.3365	4	315.8341	9.3864	0.0000
Error	1345.9288	40	33.6482		
Week	101.4891	3	33.8297	5.4687	0.0015
Week x Salinity	434.1586	12	36.1799	5.8486	0.0000
Error	742.3234	120	6.1860		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}	{17}	{18}	{19}	{20}
Salinity	Week		41.42	28.09	16.49	14.00	33.72	24.01	15.21	27.05	24.68	24.51	24.47	26.35	34.76	8.15	18.01	39.08	18.88	21.55	27.11	32.45
10	0	{1}		0.1372	0.0006	0.0002	0.7413	0.9034	0.2647	0.9234	0.8561	0.3369	0.3737	0.3143	0.4970	0.0005	0.0016	0.0201	0.9416	0.8014	0.5689	0.3145
10	1	{2}	0.1372		0.3251	0.1578	0.0854	0.7993	0.7820	0.4930	0.9070	0.9925	0.9817	0.6361	0.4512	0.6639	0.1447	0.5146	0.6210	0.9080	0.2645	0.0240
10	2	{3}	0.0006	0.3251		0.6440	0.0025	0.2397	0.9860	0.0861	0.8477	0.7077	0.6260	0.9127	0.8836	0.4769	0.9057	0.8410	0.4988	0.2515	0.4986	0.0005
10	3	{4}	0.0002	0.1578	0.6440		0.0010	0.1417	0.8645	0.6058	0.7886	0.6825	0.5118	0.9804	0.9100	0.5834	0.7027	0.8809	0.3521	0.1463	0.0070	0.1231
15	0	{5}	0.7413	0.0854	0.0025	0.0010		0.0792	0.0002	0.2481	0.5021	0.0255	0.0289	0.0292	0.1587	0.0002	0.0002	0.0005	0.7454	0.3671	0.5394	0.5895
15	1	{6}	0.9034	0.7993	0.2397	0.1417	0.0792		0.1060	0.6718	0.5354	0.9328	0.6709	0.4407	0.0676	0.3259	0.0108	0.0918	0.5970	0.9764	0.7192	0.2057
15	2	{7}	0.2647	0.7820	0.9860	0.8645	0.0002	0.1060		0.0202	0.9969	0.9997	1.0000	0.8466	0.7773	0.2294	0.8984	0.8112	0.6502	0.5068	0.6577	0.0042
15	3	{8}	0.9234	0.4930	0.0861	0.6058	0.2481	0.6718	0.0202		0.3128	0.3497	0.3923	0.7829	0.0159	0.0006	0.0019	0.5428	0.6800	0.5919	0.7824	0.7857

20	0	{9}	0.8561	0.9070	0.8477	0.7886	0.5021	0.5354	0.9969	0.3128		0.9769	0.9993	0.9555	0.9589	0.1998	0.3544	0.7415	0.9487	0.5860	0.1064	0.0048
20	1	{10}	0.3369	0.9925	0.7077	0.6825	0.0255	0.9328	0.9997	0.3497	0.9769		0.9942	0.9890	0.6479	0.7417	0.3100	0.6576	0.8408	0.9528	0.1175	0.0052
20	2	{11}	0.3737	0.9817	0.6260	0.5118	0.0289	0.6709	1.0000	0.3923	0.9993	0.9942		0.9976	0.5511	0.1450	0.7484	0.5338	0.8945	0.6991	0.7776	0.0059
20	3	{12}	0.3143	0.6361	0.9127	0.9804	0.0292	0.4407	0.8466	0.7829	0.9555	0.9890	0.9976		0.7169	0.1743	0.3322	0.9748	0.5970	0.5332	0.1199	0.3317
25	0	{13}	0.4970	0.4512	0.8836	0.9100	0.1587	0.0676	0.7773	0.0159	0.9589	0.6479	0.5511	0.7169		0.2777	0.3355	0.8034	0.8307	0.0677	0.0022	0.0002
25	1	{14}	0.0005	0.6639	0.4769	0.5834	0.0002	0.3259	0.2294	0.0006	0.1998	0.7417	0.1450	0.1743	0.2777		0.5703	0.2852	0.0168	0.3347	0.0002	0.0002
25	2	{15}	0.0016	0.1447	0.9057	0.7027	0.0002	0.0108	0.8984	0.0019	0.3544	0.3100	0.7484	0.3322	0.3355	0.5703		0.4457	0.0468	0.0106	0.1487	0.0002
25	3	{16}	0.0201	0.5146	0.8410	0.8809	0.0005	0.0918	0.8112	0.5428	0.7415	0.6576	0.5338	0.9748	0.8034	0.2852	0.4457		0.2602	0.0932	0.0035	0.0957
30	0	{17}	0.9416	0.6210	0.4988	0.3521	0.7454	0.5970	0.6502	0.6800	0.9487	0.8408	0.8945	0.5970	0.8307	0.0168	0.0468	0.2602		0.6785	0.1068	0.0011
30	1	{18}	0.8014	0.9080	0.2515	0.1463	0.3671	0.9764	0.5068	0.5919	0.5860	0.9528	0.6991	0.5332	0.0677	0.3347	0.0106	0.0932	0.6785		0.3013	0.0104
30	2	{19}	0.5689	0.2645	0.4986	0.0070	0.5394	0.7192	0.6577	0.7824	0.1064	0.1175	0.7776	0.1199	0.0022	0.0002	0.1487	0.0035	0.1068	0.3013		0.2163
30	3	{20}	0.3145	0.0240	0.0005	0.1231	0.5895	0.2057	0.0042	0.7857	0.0048	0.0052	0.0059	0.3317	0.0002	0.0002	0.0002	0.0957	0.0011	0.0104	0.2163	

c. *Balanus amphitrite*

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	3008.5977	1	3008.5977	37.5897	0.0000
Salinity	819.8744	4	204.9686	2.5609	0.0531
Error	3201.5129	40	80.0378		
Week	16.0063	3	5.3354	2.7819	0.0440
Week x Salinity	46.8965	12	3.9080	2.0377	0.0264
Error	230.1458	120	1.9179		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}	{17}	{18}	{19}	{20}
Salinity	Week		24.14	23.34	28.08	24.44	15.19	15.19	34.81	34.81	27.39	27.69	24.13	20.79	24.03	27.20	33.40	15.37	21.26	24.13	27.35	27.25
10	0	{1}		0.8401	0.8579	0.9970	0.9959	0.7674	0.8749	0.8153	0.9999	0.9985	0.9997	0.9925	0.9963	0.9690	0.9994	0.8853	0.9997	0.8945	0.7441	0.7129
10	1	{2}	0.8401		0.8382	0.9926	0.8005	0.9857	0.8446	0.7633	0.9979	1.0000	0.9998	0.9974	0.9301	0.9268	0.9998	0.8224	0.9769	0.9992	0.7425	0.7149
10	2	{3}	0.8579	0.8382		0.6300	0.8071	0.7746	0.9992	0.8702	0.9542	0.9887	0.9977	0.9917	0.9960	0.9982	0.9277	0.9470	0.9331	0.8444	0.9898	0.6800
10	3	{4}	0.9970	0.9926	0.6300		0.8790	0.8467	0.9372	0.9978	0.9793	0.9910	0.9923	0.9948	0.9977	0.9990	0.9225	0.9989	0.9360	0.8271	0.6738	0.9864
15	0	{5}	0.9959	0.8005	0.8071	0.8790		1.0000	0.5206	0.6948	0.9980	0.7043	0.8319	0.8528	0.9911	0.8359	0.8650	0.9677	0.9922	0.2231	0.0990	0.0949

15	1	{6}	0.7674	0.9857	0.7746	0.8467	1.0000		0.2769	0.5206	0.6611	0.9977	0.8039	0.8127	0.7833	0.9825	0.8345	0.9186	0.3916	0.9714	0.0920	0.0877
15	2	{7}	0.8749	0.8446	0.9992	0.9372	0.5206	0.2769		1.0000	0.8253	0.8389	0.9996	0.9124	0.8468	0.8540	0.9994	0.9476	0.5794	0.3524	0.9685	0.1694
15	3	{8}	0.8153	0.7633	0.8702	0.9978	0.6948	0.5206	1.0000		0.7941	0.8114	0.8972	0.9947	0.6954	0.7501	0.9068	0.8722	0.5471	0.3281	0.1647	0.9535
20	0	{9}	0.9999	0.9979	0.9542	0.9793	0.9980	0.6611	0.8253	0.7941		0.9296	0.3405	0.3842	0.9999	0.9954	0.9656	0.9070	0.9645	0.8116	0.7694	0.6849
20	1	{10}	0.9985	1.0000	0.9887	0.9910	0.7043	0.9977	0.8389	0.8114	0.9296		0.5496	0.4033	0.9929	1.0000	0.9875	0.9203	0.6417	0.8967	0.6930	0.5764
20	2	{11}	0.9997	0.9998	0.9977	0.9923	0.8319	0.8039	0.9996	0.8972	0.3405	0.5496		0.8633	0.9982	0.9994	0.9952	0.9636	0.8626	0.7664	0.9798	0.6035
20	3	{12}	0.9925	0.9974	0.9917	0.9948	0.8528	0.8127	0.9124	0.9947	0.3842	0.4033	0.8633		0.9925	0.9946	0.9933	0.9965	0.9589	0.8648	0.7106	0.9919
25	0	{13}	0.9963	0.9301	0.9960	0.9977	0.9911	0.7833	0.8468	0.6954	0.9999	0.9929	0.9982	0.9925		0.5386	0.5374	0.0955	0.9997	0.7868	0.5691	0.5459
25	1	{14}	0.9690	0.9268	0.9982	0.9990	0.8359	0.9825	0.8540	0.7501	0.9954	1.0000	0.9994	0.9946	0.5386		0.8346	0.0600	0.9597	0.9989	0.6618	0.6359
25	2	{15}	0.9994	0.9998	0.9277	0.9225	0.8650	0.8345	0.9994	0.9068	0.9656	0.9875	0.9952	0.9933	0.5374	0.8346		0.0147	0.9270	0.8217	0.9899	0.6353
25	3	{16}	0.8853	0.8224	0.9470	0.9989	0.9677	0.9186	0.9476	0.8722	0.9070	0.9203	0.9636	0.9965	0.0955	0.0600	0.0147		0.7208	0.4940	0.2831	0.9746
30	0	{17}	0.9997	0.9769	0.9331	0.9360	0.9922	0.3916	0.5794	0.5471	0.9645	0.6417	0.8626	0.9589	0.9997	0.9597	0.9270	0.7208		0.1926	0.0319	0.0197
30	1	{18}	0.8945	0.9992	0.8444	0.8271	0.2231	0.9714	0.3524	0.3281	0.8116	0.8967	0.7664	0.8648	0.7868	0.9989	0.8217	0.4940	0.1926		0.3104	0.1573
30	2	{19}	0.7441	0.7425	0.9898	0.6738	0.0990	0.0920	0.9685	0.1647	0.7694	0.6930	0.9798	0.7106	0.5691	0.6618	0.9899	0.2831	0.0319	0.3104		0.9651
30	3	{20}	0.7129	0.7149	0.6800	0.9864	0.0949	0.0877	0.1694	0.9535	0.6849	0.5764	0.6035	0.9919	0.5459	0.6359	0.6353	0.9746	0.0197	0.1573	0.9651	

d. *Polysiphonia* sp.

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	7730.3638	1	7730.3638	38.6161	0.0000
Salinity	2115.7417	4	528.9354	2.6422	0.0476
Error	8007.3948	40	200.1849		
Week	3460.1464	3	1153.3821	20.8882	0.0000
Week x Salinity	1701.2129	12	141.7677	2.5675	0.0046
Error	6626.0455	120	55.2170		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}	{17}	{18}	{19}	{20}
Salinity	Week		52.33	34.66	5.75	7.26	78.36	21.64	0.00	0.00	26.82	31.08	21.68	20.42	59.66	32.54	4.12	3.68	44.57	42.77	5.27	7.38
10	0	{1}		0.0629	0.0001	0.0001	0.1778	0.0004	0.0003	0.0003	0.2021	0.0126	0.0035	0.0042	0.2878	0.0038	0.0003	0.0004	0.2549	0.2551	0.0005	0.0007
10	1	{2}	0.0629		0.0151	0.0202	0.2729	0.6620	0.0674	0.0615	0.3414	0.6978	0.2582	0.3309	0.4683	0.7426	0.0779	0.0825	0.9518	0.9563	0.1219	0.1415

10	2	{3}	0.0001	0.0151		0.9794	0.9689	0.9543	0.9997	0.9925	0.9693	0.9345	0.9989	0.9674	0.7843	0.9845	0.9921	0.9926	0.1540	0.1673	0.9154	0.9932
10	3	{4}	0.0001	0.0202	0.9794		0.7632	0.9920	0.9983	0.9997	0.9515	0.9172	0.9794	0.9408	0.7702	0.9535	0.9929	0.9997	0.1707	0.1744	0.9897	0.9238
15	0	{5}	0.1778	0.2729	0.9689	0.7632		0.9298	0.9459	0.9219	0.9951	0.9616	0.9934	0.8490	0.9774	0.9736	0.9761	0.9866	0.7938	0.2721	0.9730	0.8969
15	1	{6}	0.0004	0.6620	0.9543	0.9920	0.9298		0.9962	0.9832	0.9471	0.9913	0.9841	0.9658	0.6874	0.9978	0.9000	0.9889	0.0927	0.6641	0.8942	0.9906
15	2	{7}	0.0003	0.0674	0.9997	0.9983	0.9459	0.9962		1.0000	0.9415	0.8679	0.9995	0.9789	0.6304	0.9834	0.9996	0.9891	0.0619	0.0772	0.9998	0.9990
15	3	{8}	0.0003	0.0615	0.9925	0.9997	0.9219	0.9832	1.0000		0.9251	0.8436	0.9780	0.9978	0.5987	0.9749	0.9864	0.9244	0.0567	0.0701	0.9936	0.9997
20	0	{9}	0.2021	0.3414	0.9693	0.9515	0.9951	0.9471	0.9415	0.9251		0.7437	0.6929	0.9606	0.8880	0.9336	0.9338	0.9475	0.7817	0.2861	0.9579	0.9487
20	1	{10}	0.0126	0.6978	0.9345	0.9172	0.9616	0.9913	0.8679	0.8436	0.7437		0.7499	0.9238	0.6657	0.9763	0.8643	0.8834	0.4550	0.5599	0.9115	0.9066
20	2	{11}	0.0035	0.2582	0.9989	0.9794	0.9934	0.9841	0.9995	0.9780	0.6929	0.7499		0.9949	0.7522	0.9636	0.9987	0.9859	0.2856	0.2307	0.9988	0.9803
20	3	{12}	0.0042	0.3309	0.9674	0.9408	0.8490	0.9658	0.9789	0.9978	0.9606	0.9238	0.9949		0.8918	0.9765	0.9631	0.9981	0.3450	0.3214	0.9665	0.9731
25	0	{13}	0.2878	0.4683	0.7843	0.7702	0.9774	0.6874	0.6304	0.5987	0.8880	0.6657	0.7522	0.8918		0.6665	0.2423	0.2555	0.8362	0.2751	0.7325	0.7410
25	1	{14}	0.0038	0.7426	0.9845	0.9535	0.9736	0.9978	0.9834	0.9749	0.9336	0.9763	0.9636	0.9765	0.6665		0.8938	0.9164	0.3135	0.6996	0.9811	0.9634
25	2	{15}	0.0003	0.0779	0.9921	0.9929	0.9761	0.9000	0.9996	0.9864	0.9338	0.8643	0.9987	0.9631	0.2423	0.8938		0.9829	0.0732	0.0869	0.9833	0.9931
25	3	{16}	0.0004	0.0825	0.9926	0.9997	0.9866	0.9889	0.9891	0.9244	0.9475	0.8834	0.9859	0.9981	0.2555	0.9164	0.9829		0.0770	0.0926	0.9927	0.9997
30	0	{17}	0.2549	0.9518	0.1540	0.1707	0.7938	0.0927	0.0619	0.0567	0.7817	0.4550	0.2856	0.3450	0.8362	0.3135	0.0732	0.0770		0.9817	0.0078	0.0122
30	1	{18}	0.2551	0.9563	0.1673	0.1744	0.2721	0.6641	0.0772	0.0701	0.2861	0.5599	0.2307	0.3214	0.2751	0.6996	0.0869	0.0926	0.9817		0.0110	0.0158
30	2	{19}	0.0005	0.1219	0.9154	0.9897	0.9730	0.8942	0.9998	0.9936	0.9579	0.9115	0.9988	0.9665	0.7325	0.9811	0.9833	0.9927	0.0078	0.0110		0.9749
30	3	{20}	0.0007	0.1415	0.9932	0.9238	0.8969	0.9906	0.9990	0.9997	0.9487	0.9066	0.9803	0.9731	0.7410	0.9634	0.9931	0.9997	0.0122	0.0158	0.9749	

e. *Enteromorpha clathrata*

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	1677.2854	1	1677.2854	12.3799	0.0011
Salinity	328.0342	4	82.0086	0.6053	0.6611
Error	5419.3893	40	135.4847		
Week	380.2187	3	126.7396	5.6958	0.0011
Week x Salinity	134.3335	12	11.1945	0.5031	0.9093
Error	2670.1854	120	22.2515		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}	{17}	{18}	{19}	{20}
Salinity	Week		50.89	26.65	11.75	10.71	45.15	29.99	15.52	9.33	46.19	23.34	16.10	14.37	35.57	20.82	30.28	13.33	37.03	37.70	25.28	0.00
10	0	{1}		0.9693	0.9406	0.9418	0.9876	0.9890	0.9947	0.9968	0.8054	0.9722	0.9934	0.9949	0.9986	0.9968	0.9748	0.9962	1.0000	0.9977	0.9981	0.9903
10	1	{2}	0.9693		0.9984	0.9990	0.9951	0.9995	0.8633	0.9986	0.5434	1.0000	0.9937	0.9873	0.9935	0.9583	0.9972	0.9751	0.9993	0.9990	0.9997	0.9991
10	2	{3}	0.9406	0.9984		0.9652	0.9948	0.9997	1.0000	0.9999	0.4844	0.9988	1.0000	0.9999	0.9978	0.9999	1.0000	1.0000	0.9980	0.9998	0.9921	0.9417
10	3	{4}	0.9418	0.9990	0.9652		0.9953	0.9998	1.0000	1.0000	0.4886	0.9990	0.9999	1.0000	0.9983	0.9999	0.9997	1.0000	0.9996	0.9999	0.9989	0.8549
15	0	{5}	0.9876	0.9951	0.9948	0.9953		0.9574	0.8519	0.7895	0.4589	0.8327	0.9941	0.9940	0.9988	0.9952	0.9905	0.9916	1.0000	0.9940	0.9935	0.9720
15	1	{6}	0.9890	0.9995	0.9997	0.9998	0.9574		0.9650	0.9771	0.5596	0.9996	0.9182	0.9755	0.9474	0.9983	0.9198	0.9973	0.9996	1.0000	0.9995	0.9975
15	2	{7}	0.9947	0.8633	1.0000	1.0000	0.8519	0.9650		0.9969	0.4669	0.9964	0.9989	0.9880	0.9894	0.9638	0.9997	0.9670	0.9999	0.9981	1.0000	0.9997
15	3	{8}	0.9968	0.9986	0.9999	1.0000	0.7895	0.9771	0.9969		0.4423	0.9973	0.9991	1.0000	0.9947	0.9990	0.9985	0.9995	0.8723	0.9842	1.0000	0.9996
20	0	{9}	0.8054	0.5434	0.4844	0.4886	0.4589	0.5596	0.4669	0.4423		0.0814	0.0924	0.0737	0.8949	0.5551	0.5440	0.4762	0.9747	0.4988	0.4699	0.3104
20	1	{10}	0.9722	1.0000	0.9988	0.9990	0.8327	0.9996	0.9964	0.9973	0.0814		0.9833	0.9790	0.9993	0.9999	0.9945	0.9974	0.9990	1.0000	0.9984	0.9908
20	2	{11}	0.9934	0.9937	1.0000	0.9999	0.9941	0.9182	0.9989	0.9991	0.0924	0.9833		0.8696	0.9755	0.9832	0.9915	0.9969	0.9996	0.9989	1.0000	0.9983
20	3	{12}	0.9949	0.9873	0.9999	1.0000	0.9940	0.9755	0.9880	1.0000	0.0737	0.9790	0.8696		0.9845	0.9470	0.9893	0.9995	0.9997	0.9989	0.9998	1.0000
25	0	{13}	0.9986	0.9935	0.9978	0.9983	0.9988	0.9474	0.9894	0.9947	0.8949	0.9993	0.9755	0.9845		0.9475	0.7495	0.9149	1.0000	0.9957	0.9969	0.9869
25	1	{14}	0.9968	0.9583	0.9999	0.9999	0.9952	0.9983	0.9638	0.9990	0.5551	0.9999	0.9832	0.9470	0.9475		0.9788	0.9689	0.9995	0.9997	0.9997	0.9989
25	2	{15}	0.9748	0.9972	1.0000	0.9997	0.9905	0.9198	0.9997	0.9985	0.5440	0.9945	0.9915	0.9893	0.7495	0.9788		0.9696	0.9994	0.9987	1.0000	0.9961
25	3	{16}	0.9962	0.9751	1.0000	1.0000	0.9916	0.9973	0.9670	0.9995	0.4762	0.9974	0.9969	0.9995	0.9149	0.9689	0.9696		0.9997	0.9858	0.9997	1.0000
30	0	{17}	1.0000	0.9993	0.9980	0.9996	1.0000	0.9996	0.9999	0.8723	0.9747	0.9990	0.9996	0.9997	1.0000	0.9995	0.9994	0.9997		0.9890	0.9680	0.9726
30	1	{18}	0.9977	0.9990	0.9998	0.9999	0.9940	1.0000	0.9981	0.9842	0.4988	1.0000	0.9989	0.9989	0.9957	0.9997	0.9987	0.9858	0.9890		0.9941	0.9865
30	2	{19}	0.9981	0.9997	0.9921	0.9989	0.9935	0.9995	1.0000	1.0000	0.4699	0.9984	1.0000	0.9998	0.9969	0.9997	1.0000	0.9997	0.9680	0.9941		0.9534
30	3	{20}	0.9903	0.9991	0.9417	0.8549	0.9720	0.9975	0.9997	0.9996	0.3104	0.9908	0.9983	1.0000	0.9869	0.9989	0.9961	1.0000	0.9726	0.9865	0.9534	

f. *Xenostrobus mangle*

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	81.7123	1	81.7123	8.5024	0.0058

Salinity	7.7813	4	1.9453	0.2024	0.9356
Error	384.4192	40	9.6105		
Week	51.8222	2	25.9111	8.0610	0.0006
Week x Salinity	28.1062	8	3.5133	1.0930	0.3768
Error	257.1506	80	3.2144		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}
Salinity	Week		12.82	12.82	0.00	15.84	0.00	0.00	27.88	0.00	0.00	17.24	8.70	7.13	7.13	7.13	0.00
10	0	{1}		1.0000	0.9099	0.9769	0.8471	0.9368	0.8397	0.9743	0.9014	0.9903	0.7068	0.9850	0.9202	0.9537	0.9596
10	1	{2}	1.0000		0.9316	0.7826	0.9740	0.9596	0.5140	0.9981	0.9368	0.9134	0.9574	0.9952	0.9537	0.9950	0.9743
10	2	{3}	0.9099	0.9316		0.9625	1.0000	1.0000	0.4118	1.0000	1.0000	0.9465	0.9985	0.9989	0.9992	0.9980	1.0000
15	0	{4}	0.9769	0.7826	0.9625		0.5726	0.6862	0.6905	0.9491	0.8737	0.9241	0.9138	0.9846	0.9750	0.9671	0.9310
15	1	{5}	0.8471	0.9740	1.0000	0.5726		1.0000	0.2531	1.0000	1.0000	0.8122	0.9750	0.5147	0.9138	0.8774	1.0000
15	2	{6}	0.9368	0.9596	1.0000	0.6862	1.0000		0.3191	1.0000	1.0000	0.8859	0.9846	0.9614	0.9864	0.9655	1.0000
20	0	{7}	0.8397	0.5140	0.4118	0.6905	0.2531	0.3191		0.0771	0.0521	0.4709	0.4970	0.6131	0.7881	0.5522	0.3510
20	1	{8}	0.9743	0.9981	1.0000	0.9491	1.0000	1.0000	0.0771		1.0000	0.9311	0.9996	0.9864	0.9980	0.9989	1.0000
20	2	{9}	0.9014	0.9368	1.0000	0.8737	1.0000	1.0000	0.0521	1.0000		0.8535	0.9671	0.8774	0.9655	0.9138	1.0000
25	0	{10}	0.9903	0.9134	0.9465	0.9241	0.8122	0.8859	0.4709	0.9311	0.8535		0.8501	0.9309	0.9819	0.9672	0.9113
25	1	{11}	0.7068	0.9574	0.9985	0.9138	0.9750	0.9846	0.4970	0.9996	0.9671	0.8501		0.9978	0.8857	0.9937	0.9929
25	2	{12}	0.9850	0.9952	0.9989	0.9846	0.5147	0.9614	0.6131	0.9864	0.8774	0.9309	0.9978		1.0000	1.0000	0.9881
30	0	{13}	0.9202	0.9537	0.9992	0.9750	0.9138	0.9864	0.7881	0.9980	0.9655	0.9819	0.8857	1.0000		1.0000	0.9795
30	1	{14}	0.9537	0.9950	0.9980	0.9671	0.8774	0.9655	0.5522	0.9989	0.9138	0.9672	0.9937	1.0000	1.0000		0.9583
30	2	{15}	0.9596	0.9743	1.0000	0.9310	1.0000	1.0000	0.3510	1.0000	1.0000	0.9113	0.9929	0.9881	0.9795	0.9583	

g. *Cryptosula* sp.

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	777.1317	1	777.1317	9.2808	0.0041
Salinity	1069.5146	4	267.3786	3.1931	0.0229
Error	3349.4343	40	83.7359		

Week	6.3137	3	2.1046	0.5775	0.6309
Week x Salinity	54.9406	12	4.5784	1.2564	0.2535
Error	437.2783	120	3.6440		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}	{17}	{18}	{19}	{20}
Salinity	Week		47.42	34.19	7.61	10.77	0.00	22.38	40.99	36.63	64.68	35.32	0.00	0.00	0.00	33.83	34.72	31.45	24.70	26.53	24.91	23.86
10	0	{1}		0.3861	0.2816	0.2881	1.0000	0.9955	0.9857	0.9905	0.9983	0.9920	0.9953	0.9931	1.0000	0.9218	0.8127	0.9616	0.6256	0.2964	0.2985	0.1060
10	1	{2}	0.3861		0.8072	0.7840	0.9991	1.0000	0.9978	0.9990	0.9919	1.0000	0.9997	0.9995	0.9999	0.9872	0.7857	0.9874	0.1708	0.8208	0.2303	0.1257
10	2	{3}	0.2816	0.8072		0.9969	0.8451	0.9650	1.0000	0.9997	1.0000	0.9990	0.9996	0.9790	0.9997	1.0000	1.0000	1.0000	0.2698	0.2076	0.9709	0.2962
10	3	{4}	0.2881	0.7840	0.9969		0.9987	0.9993	0.9856	0.9513	0.9985	0.9705	1.0000	1.0000	1.0000	0.9996	0.9999	0.9999	0.2320	0.1865	0.2474	0.9269
15	0	{5}	1.0000	0.9991	0.8451	0.9987		0.8131	0.9198	0.9159	1.0000	0.9951	1.0000	1.0000	1.0000	0.9999	0.9999	0.9998	0.9536	0.1483	0.2072	0.2244
15	1	{6}	0.9955	1.0000	0.9650	0.9993	0.8131		0.9863	0.9798	1.0000	1.0000	0.9993	0.9951	0.9999	1.0000	1.0000	0.9999	0.2674	0.9559	0.2794	0.2912
15	2	{7}	0.9857	0.9978	1.0000	0.9856	0.9198	0.9863		0.9052	1.0000	0.9972	1.0000	0.9998	1.0000	0.9988	1.0000	0.9933	0.2426	0.2071	0.9326	0.2498
15	3	{8}	0.9905	0.9990	0.9997	0.9513	0.9159	0.9798	0.9052		0.9989	0.9872	0.9999	1.0000	1.0000	0.9997	0.9999	0.9998	0.2551	0.2119	0.2749	0.9204
20	0	{9}	0.9983	0.9919	1.0000	0.9985	1.0000	1.0000	1.0000	0.9989		0.9863	0.9814	0.9696	1.0000	0.9873	0.9984	0.9122	0.8758	0.1817	0.2313	0.2079
20	1	{10}	0.9920	1.0000	0.9990	0.9705	0.9951	1.0000	0.9972	0.9872	0.9863		0.9899	0.9728	1.0000	1.0000	1.0000	0.9996	0.2417	0.9442	0.2551	0.2614
20	2	{11}	0.9953	0.9997	0.9996	1.0000	1.0000	0.9993	1.0000	0.9999	0.9814	0.9899		1.0000	1.0000	1.0000	1.0000	1.0000	0.2354	0.1724	0.9748	0.2651
20	3	{12}	0.9931	0.9995	0.9790	1.0000	1.0000	0.9951	0.9998	1.0000	0.9696	0.9728	1.0000		1.0000	0.9999	1.0000	1.0000	0.2181	0.1604	0.2239	0.9645
25	0	{13}	1.0000	0.9999	0.9997	1.0000	1.0000	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000		0.9507	0.9546	0.9603	0.9767	0.1844	0.2568	0.2852
25	1	{14}	0.9218	0.9872	1.0000	0.9996	0.9999	1.0000	0.9988	0.9997	0.9873	1.0000	1.0000	0.9999	0.9507		0.9684	0.9157	0.1837	0.8603	0.2188	0.1693
25	2	{15}	0.8127	0.7857	1.0000	0.9999	0.9999	1.0000	1.0000	0.9999	0.9984	1.0000	1.0000	1.0000	0.9546	0.9684		0.9884	0.1428	0.1531	0.7997	0.1210
25	3	{16}	0.9616	0.9874	1.0000	0.9999	0.9998	0.9999	0.9933	0.9998	0.9122	0.9996	1.0000	1.0000	0.9603	0.9157	0.9884		0.2121	0.1962	0.2431	0.8183
30	0	{17}	0.6256	0.1708	0.2698	0.2320	0.9536	0.2674	0.2426	0.2551	0.8758	0.2417	0.2354	0.2181	0.9767	0.1837	0.1428	0.2121		0.8403	0.9488	0.7984
30	1	{18}	0.2964	0.8208	0.2076	0.1865	0.1483	0.9559	0.2071	0.2119	0.1817	0.9442	0.1724	0.1604	0.1844	0.8603	0.1531	0.1962	0.8403		0.6194	0.8456
30	2	{19}	0.2985	0.2303	0.9709	0.2474	0.2072	0.2794	0.9326	0.2749	0.2313	0.2551	0.9748	0.2239	0.2568	0.2188	0.7997	0.2431	0.9488	0.6194		0.9451
30	3	{20}	0.1060	0.1257	0.2962	0.9269	0.2244	0.2912	0.2498	0.9204	0.2079	0.2614	0.2651	0.9645	0.2852	0.1693	0.1210	0.8183	0.7984	0.8456	0.9451	

APPENDIX 12

Summary of 2-Way Repeated Measures ANOVA and *Post Hoc* Test Results on the Effects of Fish Feed (P = Pellet, T = Trash-Fish, O = Outside Cages) and Interval Time (Minute 0, 30, 60, 90, 120) on Nutrients (NH_3-H; NO_2-N; NO_3-N & PO_4^{-3}) and Chlorophyll-*a* Concentrations

Appendix 12

Summary of 2-Way Repeated Measures ANOVA and *Post Hoc* Test Results on the Effects of Fish Feed (P = Pellet, T = Trash-Fish, O = Outside Cages) and Interval Time (Minute 0, 30, 60, 90, 120) on Nutrients (NH_3-H; NO_2-N; NO_3-N & PO_4^{-3}) and Chlorophyll-*a* Concentrations

A. Ammonia-Nitrogen (NH_3-N)

i. Flood

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	375.5556	1	375.5556	225.3333	0.0000
Feed	10.7710	2	5.3855	3.2313	0.1116
Error	10	6	1.6667		
Time	3.9229	4	0.9807	0.8586	0.5027
Time x Feed	6.0091	8	0.7511	0.6576	0.7226
Error	27.4150	24	1.1423		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}
Feed	Time		3.5714	2.3810	2.8571	2.1429	3.0952	2.6190	3.5714	4.2857	3.3333	3.8095	2.3810	1.9048	2.8571	2.1429	2.3810
P	0	{1}		0.8147	0.9224	0.8513	0.8497	0.9322	1.0000	0.8615	0.7960	0.9632	0.9475	0.7905	0.8615	0.8531	0.8895
P	30	{2}	0.8147		0.8497	0.9987	0.9224	0.7960	0.9272	0.5488	0.8988	0.8144	1.0000	0.9964	0.9531	0.9937	1.0000
P	60	{3}	0.9224	0.8497		0.9806	0.9600	0.7960	0.9683	0.8496	0.9531	0.9390	0.9844	0.9632	1.0000	0.9683	0.9531
P	90	{4}	0.8513	0.9987	0.9806		0.9701	0.9949	0.9076	0.5351	0.9616	0.8207	0.9632	0.7960	0.9928	1.0000	0.9937
P	120	{5}	0.8497	0.9224	0.9600	0.9701		0.9531	0.9531	0.7797	0.7960	0.9545	0.9849	0.9444	0.7960	0.9632	0.9783
T	0	{6}	0.9322	0.7960	0.7960	0.9949	0.9531		0.9247	0.6140	0.9224	0.8643	0.9955	0.9849	0.9632	0.9844	0.9632
T	30	{7}	1.0000	0.9272	0.9683	0.9076	0.9531	0.9247		0.6955	0.9600	0.7874	0.9444	0.8867	0.9335	0.8835	0.9217
T	60	{8}	0.8615	0.5488	0.8496	0.5351	0.7797	0.6140	0.6955		0.8092	0.5904	0.6338	0.4025	0.8074	0.5023	0.5935
T	90	{9}	0.7960	0.8988	0.9531	0.9616	0.7960	0.9224	0.9600	0.8092		0.9469	0.9632	0.8835	0.8612	0.9475	0.9390

T	120	{10}	0.9632	0.8144	0.9390	0.8207	0.9545	0.8643	0.7874	0.5904	0.9469		0.8835	0.7024	0.8988	0.7905	0.9052
O	0	{11}	0.9475	1.0000	0.9844	0.9632	0.9849	0.9955	0.9444	0.6338	0.9632	0.8835		0.9469	0.9936	0.7874	1.0000
O	30	{12}	0.7905	0.9964	0.9632	0.7960	0.9444	0.9849	0.8867	0.4025	0.8835	0.7024	0.9469		0.9701	0.9600	0.9815
O	60	{13}	0.8615	0.9531	1.0000	0.9928	0.7960	0.9632	0.9335	0.8074	0.8612	0.8988	0.9936	0.9701		0.9806	0.9815
O	90	{14}	0.8531	0.9937	0.9683	1.0000	0.9632	0.9844	0.8835	0.5023	0.9475	0.7905	0.7874	0.9600	0.9806		0.9600
O	120	{15}	0.8895	1.0000	0.9531	0.9937	0.9783	0.9632	0.9217	0.5935	0.9390	0.9052	1.0000	0.9815	0.9815	0.9600	

ii. Slack

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	1020.4082	1	1020.4082	443.3498	0.0000
Feed	55.9864	2	27.9932	12.1626	0.0077
Error	13.8095	6	2.3016		
Time	4.8753	4	1.2188	0.5997	0.6664
Time x Feed	45.9410	8	5.7426	2.8257	0.0232
Error	48.7755	24	2.0323		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}
Feed	Time		5.4762	5.9524	5.0000	6.1905	5.7143	6.1905	6.1905	5.9524	5.2381	3.5714	1.1905	3.0952	2.3810	3.5714	5.7143
P	0	{1}		0.9938	0.9123	0.9957	0.9773	0.9892	0.9986	0.9774	0.8415	0.4998	0.1229	0.3559	0.1545	0.3858	0.8415
P	30	{2}	0.9938		0.9807	0.9773	0.9773	0.8415	0.9973	1.0000	0.9898	0.5431	0.0150	0.4853	0.1326	0.4868	0.9971
P	60	{3}	0.9123	0.9807		0.9797	0.9716	0.9694	0.9894	0.9636	0.8415	0.4560	0.0324	0.3858	0.3227	0.2354	0.9296
P	90	{4}	0.9957	0.9773	0.9797		0.9938	1.0000	1.0000	0.9971	0.9891	0.5087	0.0117	0.3107	0.1107	0.5719	0.9985
P	120	{5}	0.9773	0.9773	0.9716	0.9938		0.9774	0.9985	0.8415	0.9774	0.6243	0.0182	0.3685	0.1495	0.4707	1.0000
T	0	{6}	0.9892	0.8415	0.9694	1.0000	0.9774		1.0000	0.9773	0.9807	0.4530	0.1121	0.2809	0.0987	0.4187	0.9942
T	30	{7}	0.9986	0.9973	0.9894	1.0000	0.9985	1.0000		0.9996	0.9951	0.5332	0.0131	0.4879	0.1227	0.5087	0.9996
T	60	{8}	0.9774	1.0000	0.9636	0.9971	0.8415	0.9773	0.9996		0.9716	0.4748	0.0129	0.3088	0.2791	0.4245	0.9779
T	90	{9}	0.8415	0.9898	0.8415	0.9891	0.9774	0.9807	0.9951	0.9716		0.4927	0.0263	0.3831	0.1808	0.4238	0.9144
T	120	{10}	0.4998	0.5431	0.4560	0.5087	0.6243	0.4530	0.5332	0.4748	0.4927		0.2039	0.6894	0.5768	1.0000	0.5606
O	0	{11}	0.1229	0.0150	0.0324	0.0117	0.0182	0.1121	0.0131	0.0129	0.0263	0.2039		0.2503	0.3168	0.2758	0.0168

O	30	{12}	0.3559	0.4853	0.3858	0.3107	0.3685	0.2809	0.4879	0.3088	0.3831	0.6894	0.2503		0.5454	0.9123	0.3076
O	60	{13}	0.1545	0.1326	0.3227	0.1107	0.1495	0.0987	0.1227	0.2791	0.1808	0.5768	0.3168	0.5454		0.7380	0.1255
O	90	{14}	0.3858	0.4868	0.2354	0.5719	0.4707	0.4187	0.5087	0.4245	0.4238	1.0000	0.2758	0.9123	0.7380		0.3747
O	120	{15}	0.8415	0.9971	0.9296	0.9985	1.0000	0.9942	0.9996	0.9779	0.9144	0.5606	0.0168	0.3076	0.1255	0.3747	

iii. Ebb

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	440.0113	1	440.0113	285.3603	0.0000
Feed	0.4308	2	0.2154	0.1397	0.8724
Error	9.2517	6	1.5420		
Time	6.4172	4	1.6043	1.4934	0.2355
Time x Feed	13.5147	8	1.6893	1.5726	0.1855
Error	25.7823	24	1.0743		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}
Feed	Time		3.3333	2.3810	2.8571	3.5714	3.5714	1.6667	3.3333	4.5238	3.3333	3.3333	2.8571	3.0952	3.8095	3.0952	2.1429
P	0	{1}		0.8661	0.9793	0.9997	0.9986	0.7187	1.0000	0.8721	1.0000	1.0000	0.9632	0.9608	0.9980	0.7893	0.8231
P	30	{2}	0.8661		0.5790	0.9349	0.9134	0.7003	0.9513	0.4531	0.9729	0.9562	0.8526	0.8919	0.8883	0.9257	0.7893
P	60	{3}	0.9793	0.5790		0.9969	0.9940	0.5402	0.9941	0.8453	0.9993	0.9980	1.0000	0.9608	0.9912	0.9930	0.7003
P	90	{4}	0.9997	0.9349	0.9969		1.0000	0.6254	0.9988	0.5342	0.9703	0.9930	0.9957	0.9993	0.7893	0.9985	0.8883
P	120	{5}	0.9986	0.9134	0.9940	1.0000		0.5888	0.9930	0.7047	0.7893	0.9703	0.9912	0.9980	0.9608	0.9941	0.9079
T	0	{6}	0.7187	0.7003	0.5402	0.6254	0.5888		0.5768	0.1107	0.6696	0.6262	0.7657	0.5932	0.4851	0.6717	0.5936
T	30	{7}	1.0000	0.9513	0.9941	0.9988	0.9930	0.5768		0.7931	1.0000	1.0000	0.9824	0.9950	0.9941	0.9608	0.8721
T	60	{8}	0.8721	0.4531	0.8453	0.5342	0.7047	0.1107	0.7931		0.6295	0.7228	0.7192	0.8285	0.5077	0.7871	0.3281
T	90	{9}	1.0000	0.9729	0.9993	0.9703	0.7893	0.6696	1.0000	0.6295		1.0000	0.9980	0.9998	0.9486	0.9991	0.9329
T	120	{10}	1.0000	0.9562	0.9980	0.9930	0.9703	0.6262	1.0000	0.7228	1.0000		0.9941	0.9988	0.9824	0.9930	0.9367
O	0	{11}	0.9632	0.8526	1.0000	0.9957	0.9912	0.7657	0.9824	0.7192	0.9980	0.9941		0.7810	0.9768	0.9575	0.8330
O	30	{12}	0.9608	0.8919	0.9608	0.9993	0.9980	0.5932	0.9950	0.8285	0.9998	0.9988	0.7810		0.9940	1.0000	0.7918
O	60	{13}	0.9980	0.8883	0.9912	0.7893	0.9608	0.4851	0.9941	0.5077	0.9486	0.9824	0.9768	0.9940		0.9884	0.7413

O	90	{14}	0.7893	0.9257	0.9930	0.9985	0.9941	0.6717	0.9608	0.7871	0.9991	0.9930	0.9575	1.0000	0.9884		0.8661
O	120	{15}	0.8231	0.7893	0.7003	0.8883	0.9079	0.5936	0.8721	0.3281	0.9329	0.9367	0.8330	0.7918	0.7413	0.8661	

B. Nitrite-Nitrogen (NO_2-N)

i. Flood

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	9.5681	1	9.5681	1630.7295	0.0000
Feed	0.0980	2	0.0490	8.3527	0.0185
Error	0.0352	6	0.0059		
Time	1.5761	4	0.3940	19.1210	0.0000
Time x Feed	0.4130	8	0.0516	2.5055	0.0390
Error	0.4946	24	0.0206		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}
Feed	Time		.92857	.50000	.42857	.30952	.28571	1.0357	.33333	.38095	.40476	.33333	.52381	.42857	.33333	.35714	.33333
P	0	{1}		0.0035	0.0016	0.0012	0.0009	0.1377	0.0005	0.0006	0.0007	0.0004	0.0009	0.0009	0.0004	0.0005	0.0005
P	30	{2}	0.0035		0.5481	0.8566	0.7882	0.0003	0.3174	0.8065	0.8163	0.7818	0.8281	0.5259	0.7216	0.7735	0.8657
P	60	{3}	0.0016	0.5481		0.9883	0.9738	0.0002	0.9857	0.8690	0.9739	0.9730	0.6587	1.0000	0.6657	0.9636	0.9925
P	90	{4}	0.0012	0.8566	0.9883		0.8409	0.0002	0.9739	0.9939	0.7765	0.9963	0.7050	0.9697	0.9995	0.9650	0.8281
P	120	{5}	0.0009	0.7882	0.9738	0.8409		0.0002	0.9712	0.9857	0.9697	0.9330	0.6029	0.9410	0.9978	0.9939	0.7385
T	0	{6}	0.1377	0.0003	0.0002	0.0002	0.0002		0.0003	0.0003	0.0004	0.0003	0.0006	0.0002	0.0002	0.0002	0.0002
T	30	{7}	0.0005	0.3174	0.9857	0.9739	0.9712	0.0003		0.9939	0.9893	1.0000	0.7554	0.7279	1.0000	0.9963	1.0000
T	60	{8}	0.0006	0.8065	0.8690	0.9939	0.9857	0.0003	0.9939		0.8409	0.9769	0.7735	0.8997	0.7385	0.8281	0.9978
T	90	{9}	0.0007	0.8163	0.9739	0.7765	0.9697	0.0004	0.9893	0.8409		0.9723	0.8065	0.8281	0.9118	0.7385	0.9939
T	120	{10}	0.0004	0.7818	0.9730	0.9963	0.9330	0.0003	1.0000	0.9769	0.9723		0.7088	0.9487	1.0000	0.9739	1.0000
O	0	{11}	0.0009	0.8281	0.6587	0.7050	0.6029	0.0006	0.7554	0.7735	0.8065	0.7088		0.8480	0.7315	0.7850	0.8566
O	30	{12}	0.0009	0.5259	1.0000	0.9697	0.9410	0.0002	0.7279	0.8997	0.8281	0.9487	0.8480		0.9243	0.9282	0.9907

O	60	{13}	0.0004	0.7216	0.6657	0.9995	0.9978	0.0002	1.0000	0.7385	0.9118	1.0000	0.7315	0.9243		0.8409	1.0000
O	90	{14}	0.0005	0.7735	0.9636	0.9650	0.9939	0.0002	0.9963	0.8281	0.7385	0.9739	0.7850	0.9282	0.8409		0.9996
O	120	{15}	0.0005	0.8657	0.9925	0.8281	0.7385	0.0002	1.0000	0.9978	0.9939	1.0000	0.8566	0.9907	1.0000	0.9996	

ii. Slack

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	4.2430	1	4.2430	429.6914	0.0000
Feed	0.0177	2	0.0089	0.8965	0.4564
Error	0.0592	6	0.0099		
Time	0.0762	4	0.0190	3.4149	0.0240
Time x Feed	0.0936	8	0.0117	2.0982	0.0767
Error	0.1338	24	0.0056		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}
Feed	Time		.33333	.38095	.25000	.28571	.30952	.38095	.33333	.38095	.19048	.35714	.33333	.32143	.15357	.28571	.30952
P	0	{1}		0.9337	0.8995	0.9847	0.9948	0.8320	1.0000	0.8855	0.4915	0.7191	1.0000	0.9979	0.2310	0.9954	0.9991
P	30	{2}	0.9337		0.6331	0.8830	0.9552	1.0000	0.9949	1.0000	0.2323	0.9833	0.9769	0.9916	0.0840	0.9412	0.9818
P	60	{3}	0.8995	0.6331		0.8291	0.8633	0.6510	0.8587	0.8567	0.3714	0.8199	0.9015	0.8811	0.5015	0.5900	0.8004
P	90	{4}	0.9847	0.8830	0.8291		0.9197	0.8667	0.9485	0.8987	0.6629	0.9536	0.9769	0.9471	0.2842	1.0000	0.7191
P	120	{5}	0.9948	0.9552	0.8633	0.9197		0.9258	0.9300	0.9536	0.4715	0.9883	0.9833	0.8572	0.2438	0.9833	1.0000
T	0	{6}	0.8320	1.0000	0.6510	0.8667	0.9258		0.9337	1.0000	0.1340	0.6997	0.9325	0.9411	0.0682	0.8987	0.9536
T	30	{7}	1.0000	0.9949	0.8587	0.9485	0.9300	0.9337		0.9682	0.3125	0.9794	1.0000	0.8883	0.1779	0.9769	0.9833
T	60	{8}	0.8855	1.0000	0.8567	0.8987	0.9536	1.0000	0.9682		0.1489	0.9197	0.9485	0.9681	0.3918	0.9229	0.9710
T	90	{9}	0.4915	0.2323	0.3714	0.6629	0.4715	0.1340	0.3125	0.1489		0.2427	0.4440	0.4374	0.5778	0.5091	0.3841
T	120	{10}	0.7191	0.9833	0.8199	0.9536	0.9883	0.6997	0.9794	0.9197	0.2427		0.9300	0.9817	0.1305	0.9710	0.9949
O	0	{11}	1.0000	0.9769	0.9015	0.9769	0.9833	0.9325	1.0000	0.9485	0.4440	0.9300		0.9793	0.1462	0.9847	0.9948
O	30	{12}	0.9979	0.9916	0.8811	0.9471	0.8572	0.9411	0.8883	0.9681	0.4374	0.9817	0.9793		0.1549	0.9760	0.9793
O	60	{13}	0.2310	0.0840	0.5015	0.2842	0.2438	0.0682	0.1779	0.3918	0.5778	0.1305	0.1462	0.1549		0.1613	0.1472
O	90	{14}	0.9954	0.9412	0.5900	1.0000	0.9833	0.8987	0.9769	0.9229	0.5091	0.9710	0.9847	0.9760	0.1613		0.9197

O	120	{15}	0.9991	0.9818	0.8004	0.7191	1.0000	0.9536	0.9833	0.9710	0.3841	0.9949	0.9948	0.9793	0.1472	0.9197

iii. Ebb

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	3.7144	1	3.7144	399.5244	0.0000
Feed	0.0778	2	0.0389	4.1829	0.0729
Error	0.0558	6	0.0093		
Time	0.0107	4	0.0027	0.2454	0.9096
Time x Feed	0.0696	8	0.0087	0.8016	0.6072
Error	0.2605	24	0.0109		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}
Feed	Time		.30952	.33333	.26190	.42857	.26190	.28571	.35714	.33333	.28571	.30952	.26190	.19048	.26190	.21429	.21429
P	0	{1}		0.7821	0.9991	0.6340	0.9928	0.9895	0.9409	0.9567	0.9567	1.0000	0.9692	0.9335	0.9973	0.9765	0.9636
P	30	{2}	0.7821		0.9942	0.6814	0.9780	0.9788	0.9513	1.0000	0.9409	0.9567	0.9549	0.7736	0.9882	0.9335	0.9113
P	60	{3}	0.9991	0.9942		0.7140	1.0000	0.9986	0.9848	0.9894	0.9997	0.9973	1.0000	0.8292	1.0000	0.8381	0.5744
P	90	{4}	0.6340	0.6814	0.7140		0.6326	0.6848	0.4012	0.5001	0.5822	0.7151	0.5629	0.2798	0.6580	0.4213	0.3773
P	120	{5}	0.9928	0.9780	1.0000	0.6326		0.9567	0.9636	0.9882	0.9919	0.9692	1.0000	0.9549	1.0000	0.9788	0.9269
T	0	{6}	0.9895	0.9788	0.9986	0.6848	0.9567		0.9780	0.9928	1.0000	0.9579	0.7727	0.9433	0.9919	0.9769	0.9549
T	30	{7}	0.9409	0.9513	0.9848	0.4012	0.9636	0.9780		0.7821	0.9569	0.9797	0.9433	0.6828	0.9765	0.8779	0.8527
T	60	{8}	0.9567	1.0000	0.9894	0.5001	0.9882	0.9928	0.7821		0.9797	0.9922	0.9769	0.8779	0.9835	0.9501	0.9335
T	90	{9}	0.9567	0.9409	0.9997	0.5822	0.9919	1.0000	0.9569	0.9797		0.7821	0.9567	0.9636	0.9986	0.9740	0.9769
T	120	{10}	1.0000	0.9567	0.9973	0.7151	0.9692	0.9579	0.9797	0.9922	0.7821		0.9409	0.9113	0.9925	0.9636	0.9029
O	0	{11}	0.9692	0.9549	1.0000	0.5629	1.0000	0.7727	0.9433	0.9769	0.9567	0.9409		0.9780	1.0000	0.9928	0.9797
O	30	{12}	0.9335	0.7736	0.8292	0.2798	0.9549	0.9433	0.6828	0.8779	0.9636	0.9113	0.9780		0.9155	0.7821	0.9579
O	60	{13}	0.9973	0.9882	1.0000	0.6580	1.0000	0.9919	0.9765	0.9835	0.9986	0.9925	1.0000	0.9155		0.9430	0.8426
O	90	{14}	0.9765	0.9335	0.8381	0.4213	0.9788	0.9769	0.8779	0.9501	0.9740	0.9636	0.9928	0.7821	0.9430		1.0000
O	120	{15}	0.9636	0.9113	0.5744	0.3773	0.9269	0.9549	0.8527	0.9335	0.9769	0.9029	0.9797	0.9579	0.8426	1.0000	

C. Nitrate-Nitrogen (NO_3-N)

i. Flood

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	344.8454	1	344.8454	547.4327	0.0000
Feed	0.0036	2	0.0018	0.0029	0.9971
Error	3.7796	6	0.6299		
Time	14.1887	4	3.5472	2.7981	0.0487
Time x Feed	6.8399	8	0.8550	0.6744	0.7090
Error	30.4245	24	1.2677		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}
Feed	Time		3.5714	2.2381	2.6190	3.0952	2.3810	3.3333	3.5714	2.3810	2.3810	2.1429	4.5238	2.3810	2.3810	2.6190	1.9048
P	0	{1}		0.9403	0.9010	0.9540	0.9454	0.9292	1.0000	0.9015	0.8150	0.9025	0.1922	0.8651	0.9473	0.8090	0.8043
P	30	{2}	0.9403		0.9995	0.9886	0.9869	0.9556	0.6321	0.9985	1.0000	0.9139	0.3427	0.9993	0.8711	0.9998	0.9229
P	60	{3}	0.9010	0.9995		0.8634	0.9990	0.8449	0.8090	0.9815	0.7869	0.9993	0.3344	0.9599	0.9986	1.0000	0.9953
P	90	{4}	0.9540	0.9886	0.8634		0.9851	0.7869	0.8493	0.9616	0.7015	0.9816	0.4862	0.9226	0.9906	0.4904	0.9473
P	120	{5}	0.9454	0.9869	0.9990	0.9851		0.9533	0.9015	1.0000	1.0000	0.9815	0.3681	1.0000	1.0000	0.9998	0.9404
T	0	{6}	0.9292	0.9556	0.8449	0.7869	0.9533		0.7980	0.9402	0.8363	0.9609	0.3434	0.8804	0.9708	0.6944	0.8802
T	30	{7}	1.0000	0.6321	0.8090	0.8493	0.9015	0.7980		0.8920	0.7849	0.9094	0.5265	0.5712	0.9280	0.6970	0.7759
T	60	{8}	0.9015	0.9985	0.9815	0.9616	1.0000	0.9402	0.8920		1.0000	0.9990	0.3315	1.0000	1.0000	0.9988	0.9937
T	90	{9}	0.8150	1.0000	0.7869	0.7015	1.0000	0.8363	0.7849	1.0000		1.0000	0.2535	1.0000	1.0000	0.9292	0.9993
T	120	{10}	0.9025	0.9139	0.9993	0.9816	0.9815	0.9609	0.9094	0.9990	1.0000		0.3124	0.9998	0.9599	0.9997	0.7261
O	0	{11}	0.1922	0.3427	0.3344	0.4862	0.3681	0.3434	0.5265	0.3315	0.2535	0.3124		0.3635	0.4834	0.3344	0.2864
O	30	{12}	0.8651	0.9993	0.9599	0.9226	1.0000	0.8804	0.5712	1.0000	1.0000	0.9998	0.3635		1.0000	0.9938	0.9984
O	60	{13}	0.9473	0.8711	0.9986	0.9906	1.0000	0.9708	0.9280	1.0000	1.0000	0.9599	0.4834	1.0000		1.0000	0.9540
O	90	{14}	0.8090	0.9998	1.0000	0.4904	0.9998	0.6944	0.6970	0.9988	0.9292	0.9997	0.3344	0.9938	1.0000		0.9983
O	120	{15}	0.8043	0.9229	0.9953	0.9473	0.9404	0.8802	0.7759	0.9937	0.9993	0.7261	0.2864	0.9984	0.9540	0.9983	

ii. Slack

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	178.5743	1	178.5743	176.4734	0.0000
Feed	0.2778	2	0.1389	0.1373	0.8744
Error	6.0714	6	1.0119		
Time	0.3515	4	0.0879	0.1884	0.9421
Time x Feed	1.1111	8	0.1389	0.2979	0.9596
Error	11.1905	24	0.4663		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}
Feed	Time		1.9048	2.1429	1.9048	1.7857	2.1429	1.9048	1.9048	2.3810	1.9048	2.3810	2.1429	1.9048	1.6667	1.9048	1.9048
P	0	{1}		1.0000	1.0000	0.8328	1.0000	1.0000	1.0000	0.9998	1.0000	0.9997	1.0000	1.0000	0.9221	1.0000	1.0000
P	30	{2}	1.0000		0.9733	0.9999	1.0000	0.9999	0.9552	0.9803	1.0000	0.9221	1.0000	0.9999	0.9997	0.9951	0.9988
P	60	{3}	1.0000	0.9733		1.0000	0.9927	1.0000	1.0000	0.9952	1.0000	0.9705	0.9221	1.0000	1.0000	1.0000	1.0000
P	90	{4}	0.8328	0.9999	1.0000		0.9999	0.9975	1.0000	0.9991	0.9886	0.9985	0.9999	0.9997	0.8492	1.0000	1.0000
P	120	{5}	1.0000	1.0000	0.9927	0.9999		1.0000	0.9803	0.9221	1.0000	0.7818	1.0000	0.9999	0.9998	0.9988	0.9999
T	0	{6}	1.0000	0.9999	1.0000	0.9975	1.0000		1.0000	0.9982	1.0000	0.9966	0.9999	1.0000	0.9951	1.0000	1.0000
T	30	{7}	1.0000	0.9552	1.0000	1.0000	0.9803	1.0000		0.9538	1.0000	0.9107	0.7039	1.0000	1.0000	1.0000	1.0000
T	60	{8}	0.9998	0.9803	0.9952	0.9991	0.9221	0.9982	0.9538		0.9991	1.0000	0.9951	0.9985	0.9990	0.9934	0.9969
T	90	{9}	1.0000	1.0000	1.0000	0.9886	1.0000	1.0000	1.0000	0.9991		0.9982	0.9999	1.0000	0.9803	1.0000	1.0000
T	120	{10}	0.9997	0.9221	0.9705	0.9985	0.7818	0.9966	0.9107	1.0000	0.9982		0.9803	0.9969	0.9949	0.9861	0.9979
O	0	{11}	1.0000	1.0000	0.9221	0.9999	1.0000	0.9999	0.7039	0.9951	0.9999	0.9803		0.9980	0.9982	0.9733	0.9927
O	30	{12}	1.0000	0.9999	1.0000	0.9997	0.9999	1.0000	1.0000	0.9985	1.0000	0.9969	0.9980		0.9980	1.0000	1.0000
O	60	{13}	0.9221	0.9997	1.0000	0.8492	0.9998	0.9951	1.0000	0.9990	0.9803	0.9949	0.9982	0.9980		0.9998	0.9995
O	90	{14}	1.0000	0.9951	1.0000	1.0000	0.9988	1.0000	1.0000	0.9934	1.0000	0.9861	0.9733	1.0000	0.9998		1.0000
O	120	{15}	1.0000	0.9988	1.0000	1.0000	0.9999	1.0000	1.0000	0.9969	1.0000	0.9979	0.9927	1.0000	0.9995	1.0000	

ii. Ebb

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	127.3923	1	127.3923	660.9412	0.0000
Feed	1.6553	2	0.8277	4.2941	0.0696
Error	1.1565	6	0.1927		
Time	3.6735	4	0.9184	4.7647	0.0057
Time x Feed	3.3333	8	0.4167	2.1618	0.0690
Error	4.6259	24	0.1927		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}
Feed	Time		2.1429	1.9048	1.1905	1.4286	.95238	2.3810	2.6190	1.1905	1.6667	1.9048	1.6667	1.4286	1.6667	1.4286	1.6667
P	0	{1}		0.5130	0.2756	0.5067	0.1016	0.5314	0.3910	0.2946	0.6762	0.7859	0.7636	0.5598	0.5527	0.6103	0.8333
P	30	{2}	0.5130		0.6117	0.8321	0.3042	0.3910	0.2875	0.6550	0.9097	1.0000	0.9626	0.8602	0.7859	0.9146	0.9846
P	60	{3}	0.2756	0.6117		0.9095	0.7863	0.0811	0.0195	1.0000	0.8333	0.5598	0.7673	0.7859	0.8602	0.5118	0.6762
P	90	{4}	0.5067	0.8321	0.9095		0.7669	0.2062	0.0615	0.9626	0.9069	0.7673	0.7859	1.0000	0.9626	1.0000	0.5118
P	120	{5}	0.1016	0.3042	0.7863	0.7669		0.0220	0.0046	0.5118	0.5598	0.3765	0.5031	0.6762	0.6103	0.5527	0.4970
T	0	{6}	0.5314	0.3910	0.0811	0.2062	0.0220		0.5130	0.1016	0.3752	0.5546	0.4970	0.2363	0.2939	0.2658	0.5031
T	30	{7}	0.3910	0.2875	0.0195	0.0615	0.0046	0.5130		0.0276	0.1533	0.2996	0.1757	0.1953	0.1147	0.0811	0.2062
T	60	{8}	0.2946	0.6550	1.0000	0.9626	0.5118	0.1016	0.0276		0.8790	0.6117	0.8333	0.9097	0.8906	0.7859	0.7673
T	90	{9}	0.6762	0.9097	0.8333	0.9069	0.5598	0.3752	0.1533	0.8790		0.7863	1.0000	0.9626	1.0000	0.9800	1.0000
T	120	{10}	0.7859	1.0000	0.5598	0.7673	0.3765	0.5546	0.2996	0.6117	0.7863		0.9097	0.8333	0.5118	0.8807	0.9574
O	0	{11}	0.7636	0.9626	0.7673	0.7859	0.5031	0.4970	0.1757	0.8333	1.0000	0.9097		0.9095	1.0000	0.9622	1.0000
O	30	{12}	0.5598	0.8602	0.7859	1.0000	0.6762	0.2363	0.1953	0.9097	0.9626	0.8333	0.9095		0.9843	1.0000	0.7863
O	60	{13}	0.5527	0.7859	0.8602	0.9626	0.6103	0.2939	0.1147	0.8906	1.0000	0.5118	1.0000	0.9843		0.9935	1.0000
O	90	{14}	0.6103	0.9146	0.5118	1.0000	0.5527	0.2658	0.0811	0.7859	0.9800	0.8807	0.9622	1.0000	0.9935		0.9095
O	120	{15}	0.8333	0.9846	0.6762	0.5118	0.4970	0.5031	0.2062	0.7673	1.0000	0.9574	1.0000	0.7863	1.0000	0.9095	

D. Reactive Phosphate (PO_4^{-3})

i. Flood

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	268.8889	1	268.8889	15.0179	0.0082
Feed	40.7229	2	20.3615	1.1372	0.3813
Error	107.4275	6	17.9046		
Time	44.3213	4	11.0803	0.9339	0.4610
Time x Feed	84.4750	8	10.5594	0.8900	0.5395
Error	284.7350	24	11.8640		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}
Feed	Time		.91228	6.9825	4.4211	4.4211	1.3684	.31579	1.2982	1.7544	2.1404	.94737	3.3684	.63158	5.4737	1.5088	1.1228
P	0	{1}		0.6264	0.9561	0.9693	0.9998	0.9839	0.9993	1.0000	0.9999	0.9907	0.9963	0.9250	0.9148	1.0000	0.9973
P	30	{2}	0.6264		0.7994	0.6390	0.5601	0.6252	0.7935	0.5768	0.5800	0.6621	0.7376	0.8068	0.6133	0.5908	0.6599
P	60	{3}	0.9561	0.7994		1.0000	0.8824	0.9563	0.9353	0.8645	0.7226	0.9553	0.7241	0.9645	0.9506	0.8592	0.9477
P	90	{4}	0.9693	0.6390	1.0000		0.9264	0.9675	0.9606	0.8934	0.9083	0.9704	0.9326	0.9749	0.7241	0.9478	0.9667
P	120	{5}	0.9998	0.5601	0.8824	0.9264		0.9998	0.9813	0.9907	0.9937	0.9993	0.9598	0.9999	0.8542	0.9625	0.9973
T	0	{6}	0.9839	0.6252	0.9563	0.9675	0.9998		0.9993	0.9998	0.9996	0.9960	0.9942	0.9156	0.8814	0.9999	0.9988
T	30	{7}	0.9993	0.7935	0.9353	0.9606	0.9813	0.9993		0.9985	0.9982	0.9915	0.9804	0.9996	0.8832	0.9973	0.9531
T	60	{8}	1.0000	0.5768	0.8645	0.8934	0.9907	0.9998	0.9985		0.8921	0.9997	0.8490	0.9999	0.8748	0.9344	0.9995
T	90	{9}	0.9999	0.5800	0.7226	0.9083	0.9937	0.9996	0.9982	0.8921		0.9995	0.6806	0.9998	0.7900	0.9819	0.9993
T	120	{10}	0.9907	0.6621	0.9553	0.9704	0.9993	0.9960	0.9915	0.9997	0.9995		0.9905	0.9938	0.8963	0.9997	0.9613
O	0	{11}	0.9963	0.7376	0.7241	0.9326	0.9598	0.9942	0.9804	0.8490	0.6806	0.9905		0.9913	0.8764	0.9106	0.9829
O	30	{12}	0.9250	0.8068	0.9645	0.9749	0.9999	0.9156	0.9996	0.9999	0.9998	0.9938	0.9913		0.8672	0.9999	0.9981
O	60	{13}	0.9148	0.6133	0.9506	0.7241	0.8542	0.8814	0.8832	0.8748	0.7900	0.8963	0.8764	0.8672		0.7914	0.8597
O	90	{14}	1.0000	0.5908	0.8592	0.9478	0.9625	0.9999	0.9973	0.9344	0.9819	0.9997	0.9106	0.9999	0.7914		0.9991
O	120	{15}	0.9973	0.6599	0.9477	0.9667	0.9973	0.9988	0.9531	0.9995	0.9993	0.9613	0.9829	0.9981	0.8597	0.9991	

ii. Slack

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Effect	1146.6824	1	1146.6824	114.2057	0.0000
Intercept	34.0629	2	17.0315	1.6963	0.2607
Feed	60.2430	6	10.0405		
Error	38.5709	4	9.6427	0.5288	0.7157
Time	165.5998	8	20.7000	1.1352	0.3763
Time x Feed	437.6332	24	18.2347		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}
Feed	Time		2.5965	4.7719	6.2807	3.8947	1.5439	6.9825	6.5263	4.5965	1.5088	8.7018	1.6842	7.4386	6.7018	7.5789	4.9123
P	0	{1}		0.9235	0.8935	0.7130	0.9512	0.7360	0.8955	0.8206	0.9877	0.7863	0.7366	0.8983	0.9147	0.9089	0.9557
P	30	{2}	0.9235		0.9024	0.9658	0.9359	0.9845	0.9018	0.9584	0.9542	0.9542	0.8836	0.9283	0.9770	0.9888	0.9667
P	60	{3}	0.8935	0.9024		0.9580	0.8666	0.9967	0.9417	0.9116	0.8750	0.9897	0.8067	0.9967	0.9856	0.9987	0.6839
P	90	{4}	0.7130	0.9658	0.9580		0.9059	0.9806	0.9669	0.8345	0.8788	0.9259	0.7858	0.9748	0.9779	0.8823	0.9899
P	120	{5}	0.9512	0.9359	0.8666	0.9059		0.8544	0.8475	0.8878	0.9918	0.4046	0.9667	0.8195	0.8601	0.8273	0.8308
T	0	{6}	0.7360	0.9845	0.9967	0.9806	0.8544		0.9907	0.9924	0.9039	0.9599	0.6036	0.8920	0.9334	0.9825	0.9703
T	30	{7}	0.8955	0.9018	0.9417	0.9669	0.8475	0.9907		0.9805	0.9024	0.9881	0.8236	0.9836	0.9584	0.9977	0.8789
T	60	{8}	0.8206	0.9584	0.9116	0.8345	0.8878	0.9924	0.9805		0.9464	0.9691	0.8175	0.9879	0.9544	0.9915	0.9951
T	90	{9}	0.9877	0.9542	0.8750	0.8788	0.9918	0.9039	0.9024	0.9464		0.7448	0.9985	0.8426	0.8856	0.5775	0.9669
T	120	{10}	0.7863	0.9542	0.9897	0.9259	0.4046	0.9599	0.9881	0.9691	0.7448		0.6568	0.9239	0.9738	0.7383	0.8030
O	0	{11}	0.7366	0.8836	0.8067	0.7858	0.9667	0.6036	0.8236	0.8175	0.9985	0.6568		0.8452	0.8714	0.8557	0.9359
O	30	{12}	0.8983	0.9283	0.9967	0.9748	0.8195	0.8920	0.9836	0.9879	0.8426	0.9239	0.8452		0.9758	0.9683	0.9770
O	60	{13}	0.9147	0.9770	0.9856	0.9779	0.8601	0.9334	0.9584	0.9544	0.8856	0.9738	0.8714	0.9758		0.9943	0.9552
O	90	{14}	0.9089	0.9888	0.9987	0.8823	0.8273	0.9825	0.9977	0.9915	0.5775	0.7383	0.8557	0.9683	0.9943		0.9863
O	120	{15}	0.9557	0.9667	0.6839	0.9899	0.8308	0.9703	0.8789	0.9951	0.9669	0.8030	0.9359	0.9770	0.9552	0.9863	

iii. Ebb

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	1568.0030	1	1568.0030	166.5449	0.0000
Feed	93.4539	2	46.7269	4.9631	0.0535
Error	56.4894	6	9.4149		
Time	103.8951	4	25.9738	2.1557	0.1049
Time x Feed	259.6499	8	32.4562	2.6937	0.0287
Error	289.1708	24	12.0488		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}
Feed	Time		1.1228	12.807	5.2632	5.2632	6.4211	6.2105	4.6316	12.351	6.3158	8.0702	2.7018	3.9298	5.4912	6.3158	1.6491
P	0	{1}		0.0226	0.6910	0.7640	0.7657	0.5735	0.7134	0.0189	0.6854	0.4058	0.8098	0.7434	0.7601	0.7284	0.8508
P	30	{2}	0.0226		0.2440	0.2138	0.1378	0.2421	0.2070	0.8705	0.2096	0.2186	0.0442	0.1692	0.1818	0.1601	0.0201
P	60	{3}	0.6910	0.2440		1.0000	0.9996	0.9861	0.8214	0.2686	0.9954	0.9688	0.7922	0.8808	0.9955	0.9989	0.6909
P	90	{4}	0.7640	0.2138	1.0000		0.9984	0.9378	0.9719	0.2115	0.9730	0.9470	0.8853	0.9628	0.9351	0.9918	0.7806
P	120	{5}	0.7657	0.1378	0.9996	0.9984		0.9999	0.9978	0.0990	0.9993	0.5350	0.9350	0.9914	0.9972	0.9701	0.7046
T	0	{6}	0.5735	0.2421	0.9861	0.9378	0.9999		0.9800	0.2892	0.9708	0.9638	0.7866	0.9610	0.7971	0.9993	0.7199
T	30	{7}	0.7134	0.2070	0.8214	0.9719	0.9978	0.9800		0.2195	0.9905	0.9456	0.7676	0.7890	0.9895	0.9961	0.7066
T	60	{8}	0.0189	0.8705	0.2686	0.2115	0.0990	0.2892	0.2195		0.2408	0.1441	0.0570	0.1301	0.2320	0.1530	0.0266
T	90	{9}	0.6854	0.2096	0.9954	0.9730	0.9993	0.9708	0.9905	0.2408		0.9251	0.8902	0.9757	0.9525	1.0000	0.7512
T	120	{10}	0.4058	0.2186	0.9688	0.9470	0.5350	0.9638	0.9456	0.1441	0.9251		0.6899	0.8841	0.9354	0.8033	0.4374
O	0	{11}	0.8098	0.0442	0.7922	0.8853	0.9350	0.7866	0.7676	0.0570	0.8902	0.6899		0.6688	0.9186	0.9292	0.7137
O	30	{12}	0.7434	0.1692	0.8808	0.9628	0.9914	0.9610	0.7890	0.1301	0.9757	0.8841	0.6688		0.9808	0.9886	0.7038
O	60	{13}	0.7601	0.1818	0.9955	0.9351	0.9972	0.7971	0.9895	0.2320	0.9525	0.9354	0.9186	0.9808		0.9913	0.8190
O	90	{14}	0.7284	0.1601	0.9989	0.9918	0.9701	0.9993	0.9961	0.1530	1.0000	0.8033	0.9292	0.9886	0.9913		0.8129
O	120	{15}	0.8508	0.0201	0.6909	0.7806	0.7046	0.7199	0.7066	0.0266	0.7512	0.4374	0.7137	0.7038	0.8190	0.8129	

E. Chlorophyll-*a*

i. Flood

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	52478.6465	1	52478.6465	487.8374	0.0000
Feed	2654.2126	2	1327.1063	12.3367	0.0075
Error	645.4444	6	107.5741		
Time	1759.6679	4	439.9170	3.9849	0.0128
Time x Feed	1207.4701	8	150.9338	1.3672	0.2602
Error	2649.5161	24	110.3965		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}
Feed	Time		57.230	48.210	36.447	33.213	30.883	39.367	38.607	30.037	32.493	48.413	35.407	26.783	12.497	22.533	20.123
P	0	{1}		0.5527	0.1883	0.1420	0.1154	0.2509	0.2162	0.0971	0.1315	0.3112	0.2758	0.0476	0.0011	0.0164	0.0091
P	30	{2}	0.5527		0.5288	0.5156	0.4903	0.3098	0.5302	0.4772	0.5352	0.9813	0.5728	0.3948	0.0122	0.1401	0.0882
P	60	{3}	0.1883	0.5288		0.9250	0.9653	0.9381	0.8026	0.9658	0.9668	0.6334	0.9042	0.9139	0.2950	0.7316	0.6141
P	90	{4}	0.1420	0.5156	0.9250		0.9603	0.9505	0.9215	0.9823	0.9351	0.5732	0.7996	0.9423	0.2680	0.7957	0.7254
P	120	{5}	0.1154	0.4903	0.9653	0.9603		0.9521	0.9429	0.9219	0.8521	0.5550	0.9515	0.8817	0.2909	0.7642	0.7168
T	0	{6}	0.2509	0.3098	0.9381	0.9505	0.9521		0.9302	0.9534	0.9646	0.5508	0.9636	0.8601	0.1191	0.6265	0.4910
T	30	{7}	0.2162	0.5302	0.8026	0.9215	0.9429	0.9302		0.9494	0.9516	0.6673	0.9261	0.8327	0.1264	0.6327	0.5033
T	60	{8}	0.0971	0.4772	0.9658	0.9823	0.9219	0.9534	0.9494		0.9560	0.5183	0.9695	0.7066	0.3383	0.6589	0.6570
T	90	{9}	0.1315	0.5352	0.9668	0.9351	0.8521	0.9646	0.9516	0.9560		0.5914	0.9383	0.9086	0.2604	0.7647	0.6998
T	120	{10}	0.3112	0.9813	0.6334	0.5732	0.5550	0.5508	0.6673	0.5183	0.5914		0.6546	0.3289	0.0130	0.1505	0.2218
O	0	{11}	0.2758	0.5728	0.9042	0.7996	0.9515	0.9636	0.9261	0.9695	0.9383	0.6546		0.9117	0.2106	0.7418	0.6375
O	30	{12}	0.0476	0.3948	0.9139	0.9423	0.8817	0.8601	0.8327	0.7066	0.9086	0.3289	0.9117		0.3633	0.6250	0.7209
O	60	{13}	0.0011	0.0122	0.2950	0.2680	0.2909	0.1191	0.1264	0.3383	0.2604	0.0130	0.2106	0.3633		0.4821	0.3830
O	90	{14}	0.0164	0.1401	0.7316	0.7957	0.7642	0.6265	0.6327	0.6589	0.7647	0.1505	0.7418	0.6250	0.4821		0.7813
O	120	{15}	0.0091	0.0882	0.6141	0.7254	0.7168	0.4910	0.5033	0.6570	0.6998	0.2218	0.6375	0.7209	0.3830	0.7813	

ii. Slack

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	35693.9270	1	35693.9270	416.4853	0.0000
Feed	1264.1536	2	632.0768	7.3752	0.0242
Error	514.2164	6	85.7027		
Time	622.3856	4	155.5964	1.5493	0.2200
Time x Feed	1459.4409	8	182.4301	1.8165	0.1232
Error	2410.3086	24	100.4295		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}
Feed	Time		19.143	28.763	35.130	46.240	43.407	26.450	30.960	22.810	37.503	24.250	22.480	27.653	18.960	16.957	21.750
P	0	{1}		0.9313	0.6359	0.1036	0.1802	0.9134	0.8621	0.9682	0.4732	0.9684	0.9000	0.9361	0.9821	0.9604	0.7488
P	30	{2}	0.9313		0.7199	0.3034	0.4024	0.9558	0.7813	0.9457	0.7018	0.9432	0.9690	0.8882	0.9465	0.8954	0.9744
P	60	{3}	0.6359	0.7199		0.5369	0.5770	0.8170	0.6089	0.6745	0.7706	0.7555	0.7641	0.7905	0.5933	0.5270	0.7649
P	90	{4}	0.1036	0.3034	0.5369		0.7323	0.2532	0.3417	0.1482	0.5186	0.1809	0.1559	0.2745	0.0888	0.1494	0.1467
P	120	{5}	0.1802	0.4024	0.5770	0.7323		0.3765	0.4251	0.2474	0.4698	0.3267	0.2629	0.3912	0.1651	0.1105	0.3079
T	0	{6}	0.9134	0.9558	0.8170	0.2532	0.3765		0.9454	0.8972	0.7545	0.7904	0.9499	0.8824	0.9647	0.9322	0.9766
T	30	{7}	0.8621	0.7813	0.6089	0.3417	0.4251	0.9454		0.9147	0.7069	0.9219	0.9371	0.9016	0.8863	0.8051	0.9417
T	60	{8}	0.9682	0.9457	0.6745	0.1482	0.2474	0.8972	0.9147		0.6287	0.8619	0.9677	0.9311	0.9833	0.9772	0.9906
T	90	{9}	0.4732	0.7018	0.7706	0.5186	0.4698	0.7545	0.7069	0.6287		0.6717	0.6421	0.7390	0.4978	0.4018	0.6349
T	120	{10}	0.9684	0.9432	0.7555	0.1809	0.3267	0.7904	0.9219	0.8619	0.6717		0.9739	0.9068	0.9854	0.9690	0.9864
O	0	{11}	0.9000	0.9690	0.7641	0.1559	0.2629	0.9499	0.9371	0.9677	0.6421	0.9739		0.9683	0.9728	0.9600	0.9298
O	30	{12}	0.9361	0.8882	0.7905	0.2745	0.3912	0.8824	0.9016	0.9311	0.7390	0.9068	0.9683		0.9587	0.9195	0.9774
O	60	{13}	0.9821	0.9465	0.5933	0.0888	0.1651	0.9647	0.8863	0.9833	0.4978	0.9854	0.9728	0.9587		0.8088	0.9382
O	90	{14}	0.9604	0.8954	0.5270	0.1494	0.1105	0.9322	0.8051	0.9772	0.4018	0.9690	0.9600	0.9195	0.8088		0.9355
O	120	{15}	0.7488	0.9744	0.7649	0.1467	0.3079	0.9766	0.9417	0.9906	0.6349	0.9864	0.9298	0.9774	0.9382	0.9355	

iii. Ebb

Repeated Measures ANOVA results

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	283051.8383	1	283051.8383	503.8311	0.0000
Feed	1917.8408	2	958.9204	1.7069	0.2589
Error	3370.7945	6	561.7991		
Time	2557.3725	4	639.3431	2.9726	0.0398
Time x Feed	1268.3508	8	158.5438	0.7372	0.6585
Error	5161.8265	24	215.0761		

Post hoc test results (Student – Newman – Keuls test)

			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}
Feed	Time		91.407	93.413	87.983	82.480	79.550	90.737	82.233	82.517	78.670	65.587	76.917	85.020	77.980	48.603	66.550
P	0	{1}		0.8684	0.9561	0.9740	0.9716	0.9737	0.9934	0.9659	0.9892	0.7938	0.9983	0.9663	0.9912	0.1680	0.8005
P	30	{2}	0.8684		0.9684	0.9669	0.9580	0.9795	0.9979	0.9664	0.9833	0.7418	0.9841	0.9908	0.9854	0.1397	0.7521
P	60	{3}	0.9561	0.9684		0.9671	0.9797	0.8433	0.9933	0.9574	0.9928	0.8561	0.9957	0.8315	0.9990	0.2167	0.8556
P	90	{4}	0.9740	0.9669	0.9671		0.9677	0.9739	0.9860	0.9980	0.9971	0.9159	0.9985	0.9815	0.9974	0.7095	0.9033
P	120	{5}	0.9716	0.9580	0.9797	0.9677		0.9814	0.8472	0.9964	0.9496	0.9718	0.9975	0.9945	0.9929	0.3085	0.9558
T	0	{6}	0.9737	0.9795	0.8433	0.9739	0.9814		0.9789	0.9013	0.9688	0.6273	0.9979	0.9098	0.9891	0.1673	0.7928
T	30	{7}	0.9934	0.9979	0.9933	0.9860	0.8472	0.9789		0.9997	0.9525	0.8016	0.9950	0.9989	0.9896	0.2665	0.8603
T	60	{8}	0.9659	0.9664	0.9574	0.9980	0.9964	0.9013	0.9997		0.9976	0.8817	0.9996	0.8574	0.9998	0.3350	0.9358
T	90	{9}	0.9892	0.9833	0.9928	0.9971	0.9496	0.9688	0.9525	0.9976		0.8086	0.9912	0.9971	0.9606	0.6499	0.8151
T	120	{10}	0.7938	0.7418	0.8561	0.9159	0.9718	0.6273	0.8016	0.8817	0.8086		0.6928	0.9120	0.8050	0.2294	0.9621
O	0	{11}	0.9983	0.9841	0.9957	0.9985	0.9975	0.9979	0.9950	0.9996	0.9912	0.6928		0.9970	0.9301	0.1116	0.3954
O	30	{12}	0.9663	0.9908	0.8315	0.9815	0.9945	0.9098	0.9989	0.8574	0.9971	0.9120	0.9970		0.9966	0.1395	0.8248
O	60	{13}	0.9912	0.9854	0.9990	0.9974	0.9929	0.9891	0.9896	0.9998	0.9606	0.8050	0.9301	0.9966		0.1356	0.6120
O	90	{14}	0.1680	0.1397	0.2167	0.7095	0.3085	0.1673	0.2665	0.3350	0.6499	0.2294	0.1116	0.1395	0.1356		0.3093
O	120	{15}	0.8005	0.7521	0.8556	0.9033	0.9558	0.7928	0.8603	0.9358	0.8151	0.9621	0.3954	0.8248	0.6120	0.3093	