ACOUSTIC SIGNATURES, SOUND PRODUCING MECHANISMS AND DIVERSITY OF SYNGNATHID FISHES IN MALAYSIAN WATERS

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ACOUSTIC SIGNATURES, SOUND PRODUCING MECHANIMS AND DIVERSITY OF SYNGNATHID FISHES IN MALAYSIAN WATERS

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ABSTRACT

Syngnathid fishes are known to produce sounds in various behavioural contexts. While there are very few bioacoustic studies on seahorses, pipefishes have received even less attention. Such studies are sporadic, and there are no comparative studies within the family. This PhD study reveals that the feeding click of syngnathids is composed of multiple acoustic components (high and low frequency components) whereas the distress growl of the seahorse is of a solitary low-frequency component. Another low frequency sinusoidal component named as the purr is compounded with the feeding click. The acoustic parameters (frequency and time) of syngnathid feeding click and seahorse distress growl were found to be species-specific signatures with no statistical differences among individuals of the same species. The feeding click sound is produced by two dorsal cranial bones (posteriorly, the supraoccipital bone and coronet) which is consistent throughout the *Hippocampus* genus whereas pipefishes depict varied sound producing mechanisms. In the pipefish and pipehorse, these mechanisms consist of either three cranial bones (posteriorly, the supraoccipital, 1st postcranial plate and 2nd postcranial plate) as in *Doryichthys* spp., *Trachythamphus serratus*, *Corythoichthys haematopterus*, and *Dunckerocampus dactyliophorus*, or two bones (posteriorly, the supraoccipital and 2nd postcranial plate) in the absence of the 1st postcranial plate as in *Syngnathoides biaculeatus*, or presence of a vestigial 1st postcranial plate as in *Acentronura tentaculata*. The click sound components of the seahorse can be traced to the sliding movement and forceful contact between the supraorbital bone and coronet bone (=1st postcranial plate). In *Doryichthyes* pipefishes, the click sound components are generated when the supraoccipital slides backwards, striking and pushing the 1st postcranial plate against (and striking) the 2nd postcranial plate, whereas in *Syngnathoides* pipefish, the supraoccipital rubs against the 2nd postcranial plate. Both growl and purr have the same low frequency sound but are triggered under different conditions. The growl is accompanied by intense vibration at the cheek indicating another sound producing mechanism involving possibly the pectoral girdle. The purr is hypothesised to be a result of the coronet and supraoccipital bone strike that carries the signal to the pectoral girdle through stochastic resonance. The cranial morphology and kinesis of the examined syngnathids produced acoustic signals consistent with the bone strikes that produce sharp energy spikes, or stridulation between bones that produce repeated or multimodal sinusoidal waveforms. It is hypothesized here that the extant syngnathid species either retain the ancestral three-bone mechanism or possess a derived or modified form of this model. The production of species-specific acoustic parameters in seahorses is attributed to the individual shapes and size of the coronet bone despite a common modified two-bone mechanism throughout the *Hippocampus* genus. The different mechanisms in pipefishes (i.e. either three or two participating bones) promote variability in signal acoustic shape and parameters. The variation in cranial bone morphology, cranial kinesis and acoustic signatures among syngnathid fishes reflects the adaptive evolution within the Syngnathidae which may not be influenced by its habitat preferences.

ABSTRAK

Keluarga ikan syngnathid sememangnya diketahui menghasilkan bunyi dalam pelbagai konteks kelakuan. Ketika kajian mengenai isyarat akustik kuda laut masih berada di peringkat awal, kajian isyarat akustik unduk-unduk kurang mendapat perhatian. Kajian seumpama ini bukan sahaja jarang malah tiada kajian perbandingan isyarat akustik dalam kalangan keluarga ikan ini dilakukan. Kajian ini mendapati bahawa bunyi klik ikan syngnathid terdiri daripada beberapa komponen akustik (komponen frekuensi tinggi dan rendah) manakala bunyi '*growl*' kuda laut adalah hanya terdiri daripada satu komponen frekuensi rendah. Selain daripada itu, komponen frekuensi rendah, dipanggil '*purr*' sering ditemukan bersama bunyi klik. Parameter akustik bunyi (frekuensi dan masa) klik pemakanan dan bunyi tekanan '*growl*' ikan syngnathid adalah unik dan merupakan ciri pengenalan utama spesies-spesifik dimana tiada perbezaan statistik diantara individu dalam spesies yang sama. Bunyi klik dihasilkan daripada geseran dan perlanggaran dua tulang (*coronet* dan *supraoccipital*) secara konsisten di seluruh genus *Hippocampus* manakala mekanisme penghasilan bunyi unduk-unduk adalah pelbagai. Mekanisme penhasilan bunyi unduk-unduk terdiri daripada tiga tulang, iaitu, supraoccipital, plat postcranial pertama dan plat postcranial kedua, dalam *Doryichthys* spp., *Trachyrhamphus serratus*, *Corythoichthys haematopterus*, dan *Dunckerocampus dactyliophorus* atau dua tulang (tidak termasuk plat postcranium pertama) seperti dalam *Syngnathoides biaculeatus* atau dengan plat postcranial pertama yang hadir tetapi tidak berfungsi dalam *Acentronura tentaculata*. Komponen-komponen bunyi klik kuda laut boleh dikesan daripada geseran dan pertemuan tulang supraoccipital dan tulang coronet. Bagi *Doryichthyes*, komponen-komponen bunyi klick dihasilkan apabila tulang supraoccipital bergeser, berlanggar dan menolak plat postcranial pertama kepada plat postcranial kedua, manakala bagi *Syngnathoides*, supraoccipital bergeser pada plat post postcranial kedua disebabkan ketiadaan plat postcranial pertama. Kuda laut juga didapati menghasilkan

bunyi ‘*growl*’ daripada pectoral girdle apabila diberi tekanan atau dipegang. Bunyi ‘growl’ mempunyai frekuensi yang rendah sama seperti ‘*purr*’ dan berkemungkinan dihasilkan oleh struktur mekanisme yang sama. Bagaimanapun, *growl* dan *purr* dicetuskan dan dihasilkan dibawah keadaan yang berbeza. *Purr* dihipotesiskan sebagai hasil perlanggaran tulang supraoccipital dan coronet yang melalui gemaan stotastik pectoral girdle. Kinesis kranium adalah selaras dengan penghasilan tenaga tajam (berasing atau bergabung), atau geseran antara tulang yang menghasilkan gelombang sinusoid berulang atau gelombang sinusoid pelbagai mod. It is hypothesized here that the extant syngnathid species either retain the ancestral three-bone mechanism or possess a derived or modified form of this model. Disini, ikan syngnathida dihipotesiskan mengekal mekanisme tiga-tulang kuno atau mempunyai model yang telah diterbitkan tau diubahsuaikan dari mekanisme tersebut. Penghasilan akustik spesies-spesifik kuda laut berpunca khususnya daripada bentuk dan saiz tulang coronet. Faktor tambahan punca variasi akustik unduk-unduk adalah daripada perbezaan mekanisme penghasilan bunyinya (sama ada tiga atau dua tulang yang mengambil bahagian). Variasi morfologi tulang kranium, kinesis kranium dan penghasilan bunyi spesies-spesifik ikan syngnathid berkemungkinan tinggi adalah hasil daripada proses evolusi mudah suai dalam keluarga ikan Syngnathidae dan bukan atas pilihan habitat.

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To my parents, siblings and wife, thank you for the unconditional love and support in everything I do. This dissertation is for you.

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LIST OF SYMBOLS AND ABBREVIATIONS

AS : Anecdotal record

BMNH : British Museum of Natural History

BC : By-catch

Br : Branchiostegal-ray

C : Celcius

CAS : California Academy of Sciences

CCA : Canonical Correspondence Analysis

CE : Critically Endangered

CITES : Convention on International Trade in Endangered Species

CL : Cleithrum

COR : Coronet

CR : Coral reef

cr : Cranial ridge

cs : Cranial spine

dB : Decibles

df : Degree of freedom

DD : Data deficient

DW : Deep waters

EN : Endangered

ES : Estuary

EX : Exploitation

FFT : Fast Furier Transform

FMNH : Field Museum of Natural History

GBIF : Global Biodiversity Information Facility

GCRL	:	Gulf Coast Research Laboratory Museum
HD	:	Habitat destruction or degradation
Hz	:	Hertz
IUCN	:	International Union for Conservation of Nature
KOH	:	Potassium hydroxide
KPM	:	Kanagawa Prefectural Museum of Natural History
LC	:	Least concern
MG	:	Mangrove
MNHN	:	Muséum National d'Histoire Naturelle
MPA	:	Marine Protected Area
Ms	:	Milisecond
Mya	:	Million year ago
NP	:	Nuchal plate
NS	:	Number of surveyed sites
NT	:	Near threatened
OBIS	:	Ocean Biogeographic Information System
OZCAM	:	Online Zoological Collections of Australian Museums
Pa	:	Pascal
PAR	:	Parietal
POC	:	Postcranial
POL	:	Pollution
POSTT	:	Post-temporal
Ppt	:	Parts per thousand
REC	:	Recreational activity
RG	:	Reef Guardian
ROM	:	Royal Ontario Museum

RV : River

SB : Sandy bottom

SC : Soft coral

SEM : Sesamoid bone in epaxial muscle

SG : Seagrass

SMK : Sarawak Museum of Natural History

SOC : Supraoccipital

SSC : Specific scleractinian coral

SW : Seaweed

SU : Stanford University Collection

TCM : Traditional Chinese Medicine

UBC : University of British Columbia

UMS : Universiti Malaysia Sabah

USNM : National Museum of Natural History, Smithsonian Institution

VU : Vulnerable

CHAPTER 1: INTRODUCTION

1.1 An overview of fishes and conservation of the family Syngnathidae.

Syngnathiformes is an order of ray-finned fishes that possess a narrow, elongated body made out of multiple armoured bony rings and a mouth that is tubular in shape. The order name is derived in ancient Greek which means “joined jaws”, *syn* (“together) + *gnathos* (“jaw”) and *forma* (in Latin, “shape”) (McAllister, 1968; Froese and Pauly, 2010). Five different families fall under the order Syngnathiformes such as Aulostomidae (trumpet fishes), Centriscidae (razor fishes), Fistulariidae (cornet fishes), Solenostomidae (false pipefishes) and Syngnathidae (seahorses and true pipefishes) (Kuiter, 2009; Froese and Pauly, 2010). Different models suggest that these fishes (Figure 1.1) be placed in the suborder Syngnathoides of the order Gasterosteiformes, the family of sticklebacks and its relatives. Recent evidence suggests that they are better placed in separate orders of fishes (Nelson, 2006; Kawahara *et al.*, 2008; Near *et al.,* 2013).

In the present study, only the family Syngnathidae was studied. Members of the family Syngnathidae share a common trait, fused jaw, as derived by its name in ancient Greek (mentioned above). The family comprises of four subfamilies which are seahorses (Hippocampinae), true pipefishes (Syngnathinae), flag-tail pipefishes (Doryrhamphinae), pipehorses and seadragons (Solegnathinae). They form a charismatic, unique and diverse group that is made up of 55 genera and more than 296 nominal species (Froese and Pauly, 2010; Kuiter, 2010), all of which also share a common behavioural aspect in their reproduction strategy. Members of the family are characterized by remarkable adaptations for parental care, where the female deposits eggs directly into specialized brooding area or pouch under the abdomen or tail of the male (Kuiter, 2009).

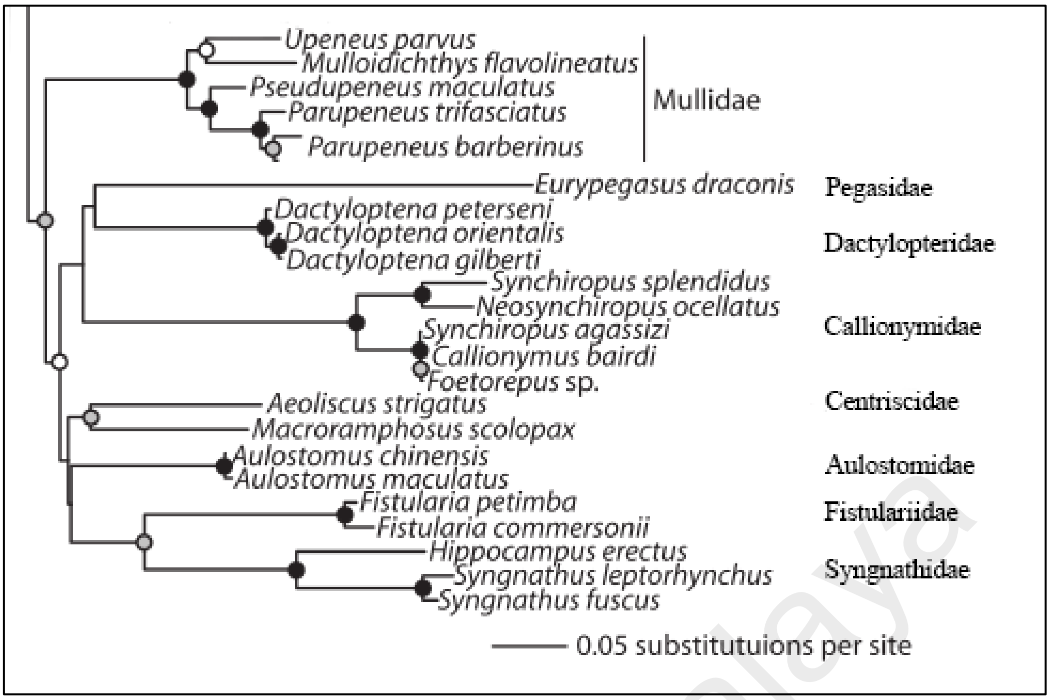

Figure 1.1: Phylogeny of the order Gasterosteiformes, adapted from Near *et al.*, 2013.

Syngnathid fishes can be found in all aquatic ecosystems and are mostly marine with some dwelling in brackish and fresh water. This group of fishes is found in temperate and tropical sea regions of the world, ranging from 50° degrees north and south of the equator (Lourie *et al.*, 2004; Kuiter, 2010). The range of these fishes can be species or group dependent, where some are found to be localised or confined to a small area, whereas others are more free ranging and widespread. Syngnathid fishes are often found in a diverse range of habitats such as seagrass beds, riverine systems, mangrove, estuaries, coral reefs, algal beds, sandy bottom, muddy bottom, rocky bottom and deeper waters (Job *et al.*, 2006; Lourie *et al.* 2004; Kuiter, 2010). However, most species occur in coastal waters and are mostly bottom dwelling, associated with reefs and seagrass meadows (Morton, 1998; Kuiter, 2010).

The taxonomy of syngnathids is confounded by synonyms, misnomers and misspellings (Lourie, 2000) rendering historical records of syngnathid biogeography unreliable. For instance, Lourie (2000), cited the problem of more than 120 species names for the seahorse subfamily (Hippocampinae), whereas the number of valid species is probably around 48 species (Vincent *et al*., 2011). In recent years, however, advances in phylogenetic studies have begun to verify the biogeography (Lourie and Vincent, 2004) and taxonomy (Lourie, 2003) of syngnathids which have provided crucial information to systematic conservation planning (Lourie and Vincent, 2004). They are typically small and cryptic and are usually associated with vegetated or other complex epibenthic habitats (Kuiter, 2009). Due to their small fins and the occurrence of semi-rigid dermal plates on their bodies, syngnathids have restricted mobility and flexibility (Kendrick and Hyndes, 2005).

Syngnathid fish are known to be important in ecological, economical, medicinal and cultural terms (Vincent *et al*., 2011). Their populations are being overfished and traded for traditional medicine, curios and aquaria. Like most countries in the region, Malaysia is an important country in the international trade in syngnathids (Perry *et al*., 2010; Martin – Smith *et al*., 2003; Martin-Smith and Vincent, 2006), facing significant conservation challenges and obligations under international trade agreements. Most syngnathids are listed as vulnerable in the IUCN Red List of Threatened Species and all seahorses are listed in the Convention on International Trade in Endangered Species (CITES) Appendix II. This is exceptional since few threatened marine or freshwater fishes have been accorded such status. Indeed, it has been shown that these charismatic creatures can serve as conservation exemplars, generating wide conservation interest and action not only for the fish but also for the environment they live in (Scales, 2010). Nonetheless, more studies are needed to update and extend the assessment of syngnathid species and their habitats.

1.2 Fish bioacoustics

Teleost fishes are known to produce acoustic sounds for intraspecific and interspecific communication through different morphological means (Fish and Mowbray, 1970; Kasumyan, 2008). The acoustic origin of sound produced by fish has been reported to be produced through stridulation, swim bladder pulsation, hydrodynamic movement, tendon vibration and air release, often creating a consistent sound pattern (Fine *et al*., 1977; Kaatz, 2002; Fine and Parmentier, 2015; Parmentier and Fine, 2016).

Stridulatory movements can be best described through the movement of any rough surface against another which would produce sound consisting of a number of short noise pulses (Burkenroad, 1930). In fishes, such sound is produced by teeth gnashing, fin spine movements, or rubbing of denticles in the pharynx (Tavolga, 1980; Hawkins, 1993). Such stridulatory sounds can be distinguished on the basis of the predominant pitch. The component frequencies produced are extended continuously from below 100 Hz and to over 8000 Hz (Tavolga, 1971).

In swim bladder vibration, a set of specialized muscles are found attached to or surrounding the swim bladder. The muscles are able to vibrate, drumming upon the swim bladder which acts as a loudspeaker in the process (Tavolga, 1980). These sounds are usually recognizable by their harmonic structure. The fundamental and predominant sound can usually be determined. If the sounds produced are a sustained call, its duration usually varies within narrow limits (Tavolga, 1971). Swim bladder pulsation creates a growling sound in fishes that has been recorded in several taxa including Holocentridae, Carapidae, Ophidiidae, Pomacentridae, Sciaenidae and Scorpaenidae (Ladich and Fine, 2006; Ramcharitar *et al.*, 2006; Parmentier and Diogo, 2006; Parmentier *et al.*, 2006a Parmentier *et al.*, 2006b; Lobel *et al.* 2010; Fine and Parmentier, 2015).

Hydrodynamic sounds are produced when the physical movements of fish in water produces a displacement of pressure wave. These sounds are evident when fish changes its swimming speed or direction abruptly (Tavolga, 1980). It has been observed that sound produced by this method are nonharmonic with the dominant frequencies extending below 100 Hz (Tavolga, 1971).

Correlation between sound production and behaviour in fishes has been grouped into six major behavioural categories: alarm sounds, territorial sounds, spawning sounds, schooling sounds, feeding sounds and echolocating sounds (Tavolga, 1971).

Alarm sounds are produced by any particular individual of some species when subjected to sufficient distress. An electrical or neurological shock can elicit a body musculature contraction so as to produce a type of detectable sound. For territorial sounds, they are sound outputs that appear when animals are in the process of defending its area, usually from intrusions. Several families of marine fishes have been observed and verified to display territorial acoustic behaviours; these include Serranidae (grouper), Holocentridae (squirrelfish), Batrachoididae (toadfish), Balistidae (triggerfish) and Pomacentridae (damselfish) (Tavolga, 1980). In spawning, sound is usually produced by the male which is associated with spawning behaviour. For example, male codfish and haddock (family Gadidae) have been reported to produce low pitch sound pulses during pre-spawning behaviour (Tavolga, 1980).

Some schooling fishes have been ascribed to pelagic fishes, such as mackerel and tuna, which produce hydrodynamic sounds through vibrations at close range. These sounds may be rhythmic subsonic vibrations produced by the fins and body of any swimming fish (Tavolga, 1971). The herring, *Clupea harengus*, has been documented produce sound during gas release. These pulsed chrips when the fish release gas through its anal pore when is scared or during ascent and descent (Wahlberg and Westerberg,

2003) Feeding sounds are often associated with stridulatory sounds of jaws and teeth, for example, the scraping of coral by a parrotfish. However, there is no evidence that fishes utilize them in any way as a method for communication (Tavolga, 1980).

The acoustic signals produced by aquatic organisms have mostly been documented in the time domain through oscillograms and also in the frequency domain by using power spectra. The combination of these two domains, i.e. presented in the form of a spectrogram, has also been employed due to the complexity of animal sounds (Foote, 2009). Common measurements taken include time (duration), amplitude, and frequency, as well as limited data on source level (Tavolga, 1971; Barimo and Fine, 1998; Wysocki and Ladich, 2002; Anderson, 2009; Locascio and Mann, 2011). However, previous literature suggests that the temporal pattern of fish sound signals, rather than the frequency spectrum, is important in the communication of aquatic organisms (Au and Hastings, 2008). The advancement of signal processing tools and their applications on bioacoustics studies have led to new insights on animal sounds and their generating mechanisms (Qian *et al.*, 2015).

1.3 Syngnathid sound production

Knowledge on the sound production in syngnathid fishes first started more than a century ago when Dufossé (1874) documented the vibrational sound made by the seahorse *Hippocampus brevirostris* during courtship. Several other studies soon followed which reported the production of finger-snapping sounds in seahorses (Gill, 1905; Fish *et al.*, 1952; Fish, 1953).

Sound production in seahorses and pipefishes (Syngnathidae) have been described in various behavioural contexts such as introduction to a new environment, during courtship and mating, inter-male competition and feeding (Fish, 1953; Fish and

Mowbray, 1970; Bergert and Wainwright, 1977; Colson *et al*., 1998; Ripley and Foran, 2008; Anderson, 2009; Anderson *et al*., 2011; Chakraborty *et al.,* 2014; Haris *et al*., 2014; Oliveira *et al*., 2014). The click originates from the coupled cranial bones of the supraoccipital and coronet (Colson *et al*., 1998; Oliveira *et al*., 2014). Two mechanisms have so far been hypothesized to explain the origin of the feeding click. The first is the stridulatory mechanism that involves the supraoccipital bone rubbing against the coronet bone (Fish and Mowbray, 1970; Colson *et al*., 1998), while the second is cavitation or the formation and sudden implosion of vapour cavities in water in the buccal cavity due to a rapid pressure change (James and Heck, 1994). Colson *et al.* (1998) tested and concluded that the clicking sound made by the seahorse was stridulatory in nature. They surgically removed the hind ridge of the supraoccipital bone and found a decrease in the clicking frequency. Although Colson and co-workers' work might support the stridulatory mechanism even through extreme manipulation to impair it, the nature of the clicking sound or how it is generated has never been investigated in detail.

Studies on the sound of seahorses have taken recent interest due to the advancement of sound-recording devices as well as signal-processing tools (Chakraborty *et al.,* 2014; Haris *et al*., 2014; Oliveira *et al*., 2014). However, there are very few works on pipefishes, the seahorse's close relative, due to their rarity and fragile nature. Pipefishes have also been reported to produce click sounds when feeding and under duress (Burkenroad, 1931; Ripley and Foran, 2007). Ripley and Foran (2007)'s work was the only and latest work on feeding strikes and click production in *Syngnathus fuscus* and *Syngnathus floridae*. They also reported interspecific differences in the sound characteristics and feeding frequency of the pipefishes under hypoxic condition.

The growls in seahorses have previously never been investigated until very recently by Oliveira and her co-workers (2014), although Kaatz (2002) had reported that it is one of two main types of sound produced in syngnathids. Anecdotal evidence of body quivering may point to the possible pulsations of the swim bladder in the seahorse (Masonjones and Lewis, 1996) as similarly reported in other species of fish (Ripley and Lobel, 2004; Amorim, 2006). Recently, Oliveira *et al*. (2014) reported a stress-associated growling sound in *Hippocampus reidi*, presumably emanating from the swim bladder although they found no evidence of extrinsic swim bladder muscles.

Most syngnathid bioacoustic studies to date feature only a single species with the exception of Colson *et al*. (1998) and Ripley and Foran (2007) with two species each. The feeding clicks of the northern pipefishes, *Syngnathus fuscus*, and dusky pipefishes, *Syngnathus floridae* were found to display interspecific differences in the temporal patterns of the signals (Ripley and Foran, 2007). However, no attempt has been made to determine whether species-specific signals exist within Syngnathidae. Therefore, in this Ph.D study, the acoustic parameters and energy components of three species of seahorses and three species of pipefishes will be measured and compared to determine if species-specific acoustics are produced.

1.4 Previous studies on the diversity of syngnathid fishes in Malaysia.

Syngnathid fishes and their habitats have been threatened worldwide by anthropogenic impacts (Vincent et al., 2011). The documentation of syngnathid fishes in Malaysian waters offers present and future studies with valuable information relating to the species richness, habitat and conservation status of these fishes. Syngnathid records within Malaysia dates back to as early as 1849 with the documentation of Hippocampus comes and Hippocampus trimaculatus in Penang waters in the Straits of Malacca (Cantor, 1849). The latter was subsequently noted by Day (1889) and Weber & and de Beufort (1922) also in Penang waters, as well as in the South China Sea. Four species of syngnathids, Doryichthys boaja (Bleeker, 1851); Ichthyocampus carce, (Hamilton, 1822); Hippichthys spicifer, (Rüppell, 1838) and Syngnathoides biaculeatus (Bloch, 1785), were soon reported by Fowler (1938) in the brackish waters ecosystems subsequently followed by Herre (1940) with syngnathids, Dorythoichthys deokhatoides, (Bleeker, 1853) and Dorythoichthys martensii, (Peters, 1869), dwelling in lentic freshwater systems in Peninsular Malaysia.

Syngnathids research and reports within Malaysia continued to grow with various reports surfacing on the freshwater species by Tweedie (1940), Johnson (1967), Mohsin (1977) and Mohsin et al., (1977). Dawson (1985) then released the first comprehensive taxonomic guide of syngnathid fishes of the world with the exception of the genus Hippocampus, recording a total of fourteen species of pipefishes from Malaysia. Syngnathid records in Malaysian waters began to pick up soon after Dawson's guide was published where syngnathids had been reported in various fish diversity checklists; however, these records remain depauperate.

The first comprehensive report on seahorses of the genus *Hippocampus*, particularly, their distribution and habitats in Peninsular Malaysia was by Choo & and Liew (2003) and soon this was followed by another report of seven species of seahorses from East Malaysia (Choo & and Liew, 2004). Choo & and Liew (2005) subsequently reported the exploitation and trading of seahorses in Peninsular Malaysia. Most recent works on the syngnathids of Malaysia include the discovery of various pygmy seahorses within Malaysia and its adjacent waters (Lourie &and Kuiter, 2008) and an in-depth study on the fisheries, large scale trading and conservation of seahorses in Malaysia and Thailand (Perry et al., 2010).

While the inclusion of syngnathid species richness and habitat preferences in Malaysian waters (Chapter 5, this thesis) may seem misplaced in the main study of syngnathid bioaccoustics, this part of the work (habitat preference) constitutes an important chapter. The reason is most studies on fish bioacoustics have been linked to behavioural, morphological and geographical differences, but there has been no attempt to link the bioacoustical aspects to habitat preference. Here, the findings from Chapter 5 have allowed me to examine the possible relationship of syngnathid habitat preference with the generated acoustic signatures and sound producing mechanisms (Chapter 6).

1.5 Research questions

The research questions that motivated the present study are as follows: -

(1) Can time-frequency analysis be used to improve our understanding of fish bioacoustics and resolve the poor resolution often associated with the usage of short time fourier transform functions using a fixed window such as sonograms?

(2) Since clicks and growls are known to produced by syngnathids, what are their signal feature differences, mechanisms of generation and significance?

(3) If assuming that sounds are produced for communication, do syngnathids produce individual- and species-specific acoustic signatures?

(4) Similarly, do seahorses and pipefishes differ in their sound producing mechanisms but share similarities as a family?

(5) Are the acoustic signal parameters (frequency and duration) in syngnathids influenced by the sound producing mechanism?

(6) Since the propagation of sound waves is affected by the medium and objects, are the acoustic signals produced by syngnathids related to their habitat type or environment?

(7) If the generated sounds bear unique signatures, can sound signatures and their generating mechanisms in Syngnathidae provide useful taxonomic and phylogenetic information?

(8) What are the current threats to the conservation of Syngnathidae?

1.6 Significance of study

This Ph.D study was carried out to contribute to new knowledge and understanding of the acoustic capabilities of fishes from the family Syngnathidae. While anecdotal reports of syngnathid sound production in various behavioural context were present, detailed documentation on its signal and associated sound producing mechanism are limited. Furthermore, previous anecdotal evidence of body quivering points to the production of another sound, possibly pulsations of the swim bladder in the seahorse (Masonjones and Lewis, 1996), as similarly reported in other species of fish (Ripley and Lobel, 2004; Amorim, 2006). This study also investigates the origin of the stress-associated growling sound reported by Oliveira *et al.* (2014) who were not able to determine the mechanism of production. Additionally, no further attempts (with the exception of Ripley and Foran (2007)) were made to compare the signal parameters and the sound producing mechanisms with other syngnathid fishes. This study explores the sound produced in the behavioural ecology of syngnathid fishes and their sound producing mechanisms to provide useful taxonomic and phylogenetic insights. Therefore, a detailed study will provide better understanding of the acoustic signal and its sound producing mechanism so as to fill up the knowledge gaps pertaining to the bioacoustics capabilities of these fishes. By collating data from previous and current work on the distribution and habitats of syngnathids, this study will provide the current threats to the conservation of these fishes.

1.7 Objectives of study

The main objectives of this study are: -

(1) To study sound production in syngnathid fishes in relation to feeding and stress using time-frequency analysis as a new method in the study of bioacoustics.

(2) To determine the intra- and interspecific differences in acoustic signal parameters (frequency and duration) among selected syngnathid species.

(3) To document and compare the sound producing mechanisms of several syngnathid species.

(4) To collate the syngnathid records and document their diversity, distribution and habitats in Malaysian waters.

(5) To determine whether syngnathid acoustic signals (or their production mechanisms) are related to their habitat type.

(6) To assess the current threats to the conservation of syngnathid fishes in Malaysian waters.

1.8 Investigations to address the study objectives

The following investigations were carried out to address the above objectives: -

(1) Chapter 3 and 4: Recording, analysis and comparison of syngnathid acoustic signals in relation to feeding and stress (Objective 1 and 2).

(2) Chapter 3 and 4: Digital scanning, clearing and staining and description of syngnathid sound producing bones (Objective 3).

(3) Chapter 5 and 6: Relationship between syngnathid habitat preference, its acoustic signal and its mode of generation (Objective 4, 5 and 6).

CHAPTER 2: METHODOLOGY

2.1. Syngnathid diversity and distribution of Malaysia

In this study, syngnathid diversity was documented through several approaches which involved the examination of collected specimens, museum specimens, records from published journals and online databases. The survey and documentation covered a total of 55 sites from 35 localities in Malaysian waters (Figure 2.1). Sites of collected and recorded syngnathids included freshwater, estuarine and marine ecosystems.

Opportunistic samplings were employed to sample the syngnathids in their known habitats which occurred in 35 sites from 27 localities (Figure 2.1). The fish were caught using scoop nets, gill nets, push nets, beam trawls and commercial otter trawls. In clear water habitats, such as coral reefs, in-water observations of syngnathids using SCUBA were carried out, with limited collections. Photographs and video recordings were made as samples for identification, especially within marine protected areas where extraction of biological specimens is strictly prohibited by law. Specimens were preserved in 80% alcohol or air dried before species identification. Details of habitats of the collected or observed fishes were recorded wherever possible.

2.1.1 Species identification

The specimens were identified using the keys of Lourie *et al*. (2004) and Lourie and Kuiter (2008) for seahorses and Dawson (1985) and Kuiter (1998) for other syngnathid fishes. Only positively identified syngnathids, especially those from photographs and videos, were included in the results.

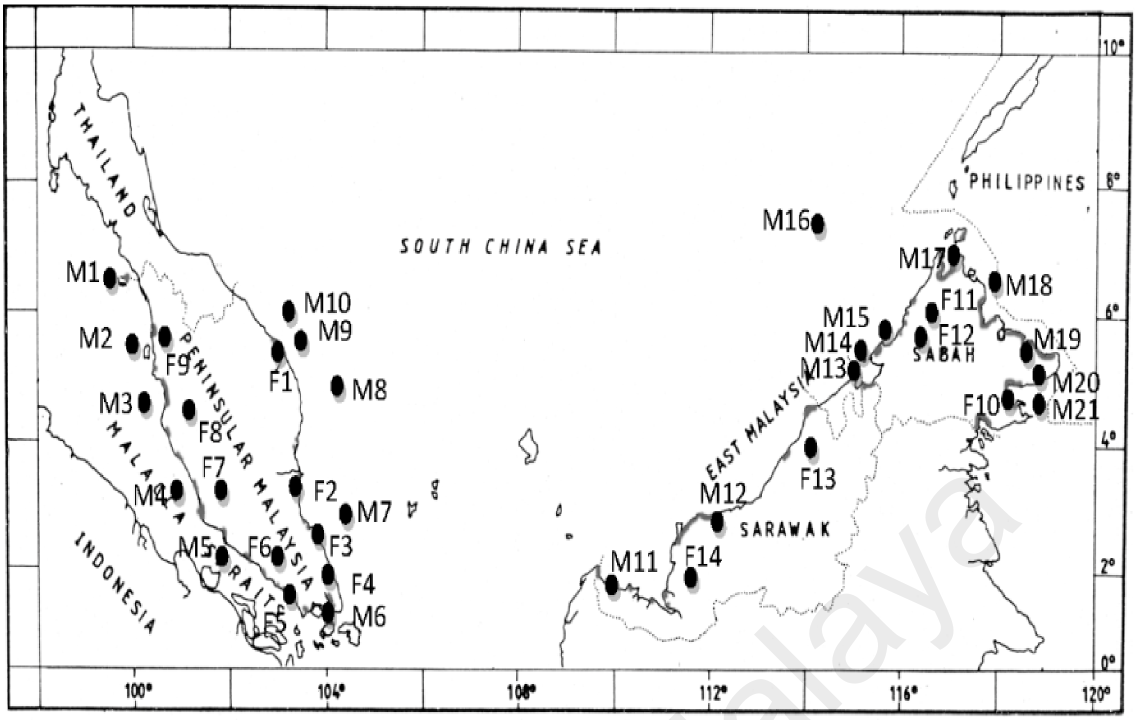

Figure 2.1: Main distribution sites of Malaysian syngnathids: Freshwater: Kuala Berang, Setiu, Tersat (F1), Endau – Rompin, Kahang River, Kg. Ayer Puteh (F2), Mawai, Kota Tinggi (F3) Johor River, Perepat River (F4), Kuala Skudai (F5), Muar, Simpang Renggam (F6), Klang (F7), Tapah (F8), Perak River, Kuala Sepetang (F9), Tawau (F10), Sulaman River (F11), Inanam River (F12), Kejin River, Niah River (F13), Rajang River, Kuap River (F14). Marine water: Payar, Langkawi (M1), Penang (M2), Sembilan Islands, Pangkor (M3), Carey Island, Morib, Kuala Bernam (M4), Tanjung Tuan, Malacca (M5), Pulai River, Pisang Island (M6), Mersing Islands (M7), Tenggol Island (M8), Kapas Island (M9), Perhentian, Redang, Bidong Island, Merang (M10), Santubung (M11), Mukah (M12), Labuan (M13), Tiga Islands (M14), Tunku Abdul Rahman Park, Kota Kinabalu (M15), Spratly Islands (M16), Banggi Islands (M17), Sugud Islands (M18), Sandakan (M19), Lahad Datu (M20), and Semporna Islands (M21).

2.1.2 Museum and databases list

The museums and databases where data were taken from included BMNH (Natural History Museum, London, Great Britain, formerly British Museum of Natural History); CAS (California Academy of Sciences, San Francisco, U.S.A.); FISHWISE

(Universal Fish Catalogue); FMNH (Field Museum of Natural History, Chicago, U.S.A.); GBIF (Global Biodiversity Information Facility); GCRL (Gulf Coast Research Laboratory Museum, Ocean Springs, Mississippi, U.S.A.); KPM (Kanagawa Prefectural Museum of Natural History, Kanagawa, Japan); MNHN (Muséum National d'Histoire Naturelle, Paris, France); OBIS (Ocean Biogeographic Information System); OZCAM (Online Zoological Collections of Australian Museums, Australia); RG (Reef Guardian, Sabah, Malaysia); ROM (Royal Ontario Museum , Toronto, Canada); SMK (Sarawak Museum of Natural History, Malaysia); SU (Stanford University Collection, deposited in California Academy of Sciences, San Francisco, U.S.A.);UBC (University of British Columbia, Vancouver, Canada); UMS (Universiti Malaysia Sabah, Kota Kinabalu, Malaysia) and USNM (National Museum of Natural History, Smithsonian Institution, Washington D.C., U.S.A.). Data from museums and databases were examined and synonyms and species of unlikely occurrence were excluded from the study. No attempt was made to verify species identification of specimens kept outside Malaysia.

The number of collected syngnathids and sites of occurrence were recorded. Abundance of syngnathids was neither estimated nor compared among sites because of the different sampling methods, unequal samplings and varied catch effort.

2.1.3 Threat assessment

Syngnathid fishes were evaluated for threats detrimental to their conservation and survival. All species depending on the available information were assessed for the following potential threats: exploitation (EX), by-catch (BC), habitat destruction or degradation (HD), pollution (POL), and recreational activities (REC). Exploitation pertains to syngnathids targeted in the fishery; by-catch refers to syngnathids incidentally caught; habitat destruction or degradation are due to development, reclamation, fishing methods and other human activities; pollution includes all types including sedimentation; and recreational activities include diving, snorkeling and nature travel activities. The threat assessment was based on present observations of these activities in the localities of the syngnathids, Department of Environment reports and published literature. The level of threat to species was however not completely assessed due to deficient data, except those already assessed by IUCN and Chong et al. (2010).

2.2 Animal husbandry

2.2.1 Animal collection

All live fishes were purchased from local aquaria dealers. Fishes were treated with freshwater bath for marine species and saltwater bath for freshwater species for a couple of minutes and isolated for a week before introduction into the main housing tanks. Syngnathids were kept, acclimatised and maintained in aquaria for at least 3 months before the experiments. All fishes were checked thoroughly for signs of illness or injuries before they were used for the experiments.

2.2.2 Animal care

2.2.2.1 Saltwater tank

Seahorses and marine pipefishes were kept in 30.0 ppt salinity water in a plastic aquarium (160 cm x 100 cm x 50 cm) without bottom substrate supplied with artificial plants acting as holdfast. The aquaria was kept inside a fish hatchery and exposed to dim natural sunlight. Water temperature was kept at 26 °C ± 2 °C. All unconsumed food and waste were siphoned along with 10.0% daily water change.

The fish were fed three times daily with mixed enriched brine shrimps, daphnia, live poecilid fish larvae and gut loaded glass shrimps. Glass shrimps were gut loaded with marine food pellets for carnivorous fishes.

2.2.2.2 Freshwater tank

Freshwater pipefishes, *Doryichthys martensii* and *Doryichthys deokhatoides* were kept in glass aquarium (60 cm x 60 cm x 60 cm) with sparse aquatic plants acting as refuge for the fishes. The aquria was kept inside fish hatchery and exposed to dim natural sunlight. Water temperature was maintained at 26.0 ± 2.0 °C. All waste and unconsumed food materials were siphoned along with a 10.0% daily water change.

Pipefishes were fed twice daily with mixed daphnia, glass shrimp larvae and enriched brine shrimp nauplii. Brine shrimp nauplii were enriched with blended instant cereal.

2.2.3 Animal ethics

Experimental protocols involving the live syngnathid fishes were approved by the Institutional Animal Care and Use Committee, University of Malaya (UM IACUC), with Ethics References No. ISB/14/08/2012/ALCO(R). No fish deaths resulted from the experiments, and fish were returned following the experiment to their aquaria housed in the University of Malaya’s Marine Culture Unit.

2.3 High speed photography recording of feeding

The fish was placed in a transparent glass tank (30.0 cm x 10.0 cm x 26.0 cm) in order to record feeding attempts using a high-speed camera (Eosens CL high-speed CMOS) at 2000 frames per second. Ten glass shrimps were placed in the transparent glass tank with the fish prior to recording. Any feeding attempts that occurred during the resting stage prior to or following the production of clicks were extracted from the video recordings. The duration of the feeding attempt to the end of a successful strike was compared to the duration of acoustic recordings made.

2.4 Sound producing mechanism in syngnathids

2.4.1 Microtomography

Cranial morphology of the specimens was captured and analysed using microtomography (SkyScan 1172) high-resolution microtomography) services by Nuclear Agency Malaysia. The heads of two specimens of each species were examined. The head was mounted onto a holder and placed into the microtomographic scanner to fit into the field of view. Scan layers were set to 10 µm on a 180° rotation to acquire frames. The image frames were collated and reconstructed using a 3D imaging software (CTvox) to determine the cross section and morphological structure of the head bones.

2.4.2 Clearing and staining

The cranial anatomy of alcohol-preserved specimens was further examined by using a clearing and double-staining method for bone and cartilage (Dingerkus and Uhler, 1977). Three specimens of each species were cleared in 35 ml saturated sodium borate, 65 ml distilled water and trypsin powder for 24 hours; and then double stained in 100 ml 1.0% KOH solution with 1.0 mg Alizarin Red stain for 48 hours, and 30 mg Alcian Blue, 60 ml ethanol and 40 acetic acid for 36 hours, respectively. Because the whole head could not be viewed in the same field under the microscope, parts of the same head were photographed under a stereo microscope (Leica M125) attached to a digital camera system (Leica MC170 HD). All photos were then stitched together to reproduce the whole fish head using the Leica Application Suite (LAS) (version 4.3).

2.4.3 Terminologies used

Interpretations and descriptions of histological and microtomographic images are primarily based on the terminologies used by Leysen *et al*. (2011) for pipefishes and Ginsburg (1937) and Azzarello (1989) for seahorses.

2.4.4 Sound recording set-up

Experiments with live marine fishes were conducted in an acoustic dampened tank (160.0 cm x 100.0 cm x 45.0 cm) filled with seawater, while experiments with freshwater fishes were conducted in smaller acoustic dampened tanks (60.0 cm x 45.0 cm x 40.0 cm) filled with freshwater. Both tanks were lined on the inside with 1-inch polystyrene foam and air-filled packing wraps and filled with bottom sand to reduce resonance and reflection, following the method used by Wysocki and Ladich (2002). The tank was placed on a 2-inch foam block to further reduce resonance from background noise.

Sound recordings of individual fish were recorded one at a time. The fish was confined in a fabricated plastic mesh cage (30.0 cm x 20.0 cm x 45.0 cm; 0.3 cm mesh opening) placed inside the acoustic tank and allowed to acclimatized for 48 hours before sound recordings were made. The marine fishes were maintained inside the net cage and fed with glass shrimp, *Macrobrachium lanchesteri* and live poecilid fish larvae whereas, freshwater fishes were not confined in any mesh cage inside the tank, and were fed with live brine shrimp nauplii. All mechanical filters and heaters were shut down two hours prior to the experiment.

Audio signals emitted during feeding were recorded using a hydrophone (Cetacean Research Technology, Model C55-F2-LAB) with a frequency range of 0.006 to 203 kHz. The hydrophone was omnidirectional with a sensitivity of -165 dBre 1V/µPa; preamplifier gain: 20 dB connected to a compact flash recorder (Fostex FR-2 24 bit/ 192 kHz). The hydrophone was placed at mid-water level at the centre of the recording tank, 20cm away from the mesh cage. The calculated minimum attenuation distance was 30.1 cm for the saltwater tank and 21.9 cm for the freshwater tank with a minimum resonant frequency of 2353.2 Hz for saltwater and 2802.8 Hz for freshwater. Both distances were below the calculated levels based on the equations of Akamatsu *et al.* (2002). Background noises were pre-recorded as control before the experimental recordings were performed. All recordings were examined for the sound signals and isolated for independent analysis. Individual pulses were identified from the start of the visible incremental energy to the tail end where the energy fades off.

To induce and record the growl sound, the seahorses were subjected to distress by placing it inside a muslin net cage (size 10.0 cm x 15.0 cm) underwater in the acoustic tank. Recordings of sounds emitted were similarly made as described above. In both the click and growl experiments, the top of the confinement cage was exposed to allow for observations of fish behaviour.

2.5 Data analysis

2.5.1 Time-frequency analysis

Time-frequency representation of signal is important in a bioacoustic study as it allows for the spectral content of a signal to be traced over time. Conventionally, time-frequency analysis is performed by taking several local Fourier transformations on the sound sample using fixed window size. The resulting spectral at each time instant gives the spectrogram. However, due to Heisenberg-Gabor inequality, one cannot achieve high temporal resolution and frequency resolution at the same time. For example, if a wider window is used in the transform, one obtains good frequency resolution at the cost of poorer time resolution.

To resolve the limitation of Fourier transform, an alternative time-scale representation based on wavelet transform was introduced. Wavelet transform analysis has been widely applied in bioacoustic study as a signal processing and recognition or classification tools (Selin *et al*., 2007; Ng *et al*., 2011; Martinez *et al*., 2013). The continuous wavelet transform of a signal *X(s)* is defined as (Daubechies, 1992)

$$W(a,t)=\frac{1}{\sqrt{a}}\int \psi\left(\frac{t-s}{a}\right)X(s)ds$$

where the localized function $\psi(\cdot)$ is called the mother wavelet, a is the scale parameter and t is the time shift parameter. Scale parameter a functions to dilate ($a > 1$) and compress ($a < 1$) the mother wavelet ψ, hence creating a time-varying multiscale bases. The scale parameter is inversely proportional to frequency. In other words, small scale refers to high frequency and vice versa. There are a variety of mother wavelet families such as Mexican hat, Daubechies and Morlet wavelet, each with their own properties (Daubechies, 1992). The choice of mother wavelet depends on the local properties of the analyzed signal. Generally, a mother wavelet of high regularity should be used when the analyzed signal is differentiable and vice versa. The corresponding 'energy distribution' to spectrogram is called wavelet scalogram, and is defined as $S(a,t)=|W(a,t)|^2$ (Rioul, 1992). This time-scale representation has an equivalent time-frequency expression that can be obtained by scale-frequency conversion $a=\frac{f_0}{f}$, where f_0 is the central frequency of the mother wavelet ψ. In contrast to fixed window sized of spectrogram, scalogram has varying window size at different scales thus preserving the time and frequency resolution.

Before the sound data were analyzed, the background signal peak at 30 Hz was removed using wavelet detrending. The raw signal was decomposed into twelve dyadic scale levels using multiresolution decomposition with Daubechies N =5 mother wavelet. Then the 30 Hz signal was reconstructed based on scale level 9 decomposition, and this so-called band-passed interference signal was removed from the raw signal to give the detrended signal.

The wavelet multiresolution was carried out using the Matlab Time-Frequency toolbox developed by Auger *et al*. (1998). As for the wavelet scalogram, Morlet wavelet of 50 half-length was used in the analysis. The scalogram was calculated over 128 analyzed voices and was bounded between 0.001-0.04 Hz normalized frequencies. Squared magnitude of continuous wavelet transform with threshold value over 0.5 was depicted in the scalogram. The energy spectral density that gives the frequency marginal of the scalogram was plotted side by side with the scalogram.

Sound characteristic data collected includes dominant frequency and sound duration for all sound recorded. Dominant frequency is the frequency value with the highest energy spectral density time whereas sound duration is the temporal length of the sound and is measured from the spectral form.

Assuming that the decaying oscillatory signal follows the exponential function $y=e^{-t/\tau}$, then τ, also known as the time constant (s^{-1}) which indicates how fast the signal's amplitude decays to 1/e can be estimated (Provencher, 1976). τ was estimated by an algorithm that first identified the local peaks of the signal and then fitting the peak coordinates to the exponential equation using the Matlab curve fitting function

(MATLAB 8.5 and Statistic Toolbox 10.0, The Mathsworks, Inc., Massachusetts, United States).

2.5.2 Fast fourier transformation sound analysis

Traditionally, the analysis of fish sounds uses an oscillogram, sonogram and average power spectra (Tavolga, 1971; Ripley and Foran, 2007; Chakraborty *et al*., 2013). For the purpose of comparing these traditional methods against the time-frequency analysis, the recorded sounds of one species of seahorse, *Hippocampus comes,* were analysed using an oscillogram, spectrogram and average power spectra. These were derived from the sampled data through the Fast Fourier Transform (FFT) using MATLAB analytical software (MATLAB 8.5 and Statistic Toolbox 10.0, The Mathsworks, Inc., Massachusetts, United States). All power spectra were calculated from 44.1 kHz- sampled data using a 1,024 point FFT with a Hanning window. The generated output of the FFT was compared with the output from time-frequency analysis of the exact sound recording of the same seahorse.

2.5.3 Vibration analysis

In the aquarium, the seahorse's body at its dorsal fin was laterally grasped gently between the thumb and index finger to induce stress and stimulate the production of growls. A fine vibrational probe connected to a PCB Piezotronics uni-axial accelerometer (Model 352B10), held by the other hand, was used to measure the growl vibrations by gently contacting the seahorse's body. Growl vibrations were measured at ten pre-selected points (Figure 2.2), one point after another from head to tail, for twelve seconds at each point. In a virtual instrument application programme (DASYLab, Data Acquisition System Laboratory, DasyTec USA, Amherst, New Hampshire), the sampling rate used was 25600 samples s^{-1} with block size of 16384. This yielded frequency resolution of 1.5625 Hz and 0.64 seconds of time record length to capture every response signal generated by the seahorse. Twenty averages were taken at each measurement point and the experiment was repeated for two sets of measurement. The time response signals were displayed and further processed in DASYLab v10.0 to obtain the maximum vibration amplitude in the unit of acceleration (g) and the standard deviation. Spectrum in frequency domain of response signals for all measurement points were obtained through Fast Fourier Transformation (FFT).

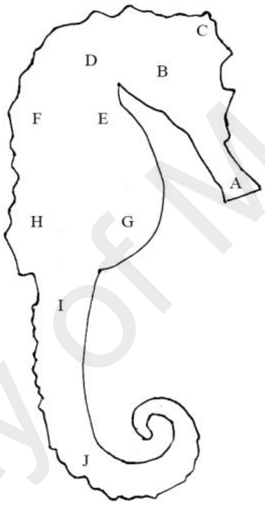

Figure 2.2 Accelerometer recording points on a seahorse.

2.6 Statistical analysis

2.6.1 Sound characteristics among species

Sound characteristic values were tested for normality and homoscedascity using Shapiro-Wilk's test. The mean of the sound characteristic values was calculated for each fish. Differences in measured sound characteristics among species were tested using Kruskal-Wallis non-parametric test. The Mann-Whitney U-test was used to test the differences of the sound characteristics of the low frequency component between the congeneric species *Doryichthys martensii* and *Doryichthys deokhatoides.* Statistical tests were carried out using Statistica 10.0 software (StatSoft Inc., Tulsa, Oklahoma, USA).

2.6.2 Relationship between syngnathid habitat preference and signal characteristics.

Syngnathid habitats (coral reef; freshwater; mangrove; sandy bottom; muddy bottom; seagrass; sponges; seaweed) were ranked according to the following habitat features: complexity, depth, salinity and presence of hard surfaces. Ranking for each feature was based on the following order: Complexity: 1 = least complex, 2 = low complexity, 3 = averagely complex, 4 = high complexity, 5 = most complex; depth: 1 = shallow (< 40 meters), 2=deep (> 40 meters); salinity: 1 = freshwater, 2 = brackish water, 3 = sea water; presence of hard surfaces: 1 = none or limited hard surfaces, 2 = prevalent hard surfaces). Principle component analysis (PCA) was carried out to relate six syngnathid species (Dd = *Doryichthys deokhahtoides*; Dm = *Doryichthys martensii*; Hb = *Hippocampus barbouri*; Hc = *Hippocampus comes*; Ht = *Hippocampus trimaculatus*; and Sb = *Syngnathoides biaculeatus*) to their feeding click acoustic parameters, namely, minimum frequency, maximum frequency, minimum duration and maximum duration. The additional data of six other syngnathid species were obtained from the following sources: He = *Hippocampus erectus* (Colson *et al*., 1998); Hk = *Hippocampus kuda* (Haris *et al*., 2015); Hr = *Hippocampus reidi* (Oliveira *et al*., 2015); Hz = *Hippocampus zosterae* (Colson *et al*., 1998); Sf = *Syngnathus fuscus* (Ripley and Foran, 2007); and Sr = *Syngnathus floridae* (Ripley and Foran, 2007). Canonical correspondence analysis (CCA) was carried out to determine the relationship between the number of syngnathids collected with habitat features (complexity, depth, salinity, presence of hard surfaces) and type of habitats (coral reef; freshwater; mangrove; sandy bottom; muddy bottom;

seagrass; sponges; seaweed). Both PCA and CCA were performed using CANOCO 4.5 software (Ter Braak and Smilauer, 2002). Additionally, a Spearmann's rank correlation coefficient test was carried out between habitat (coral reef; freshwater; mangrove; sandy bottom; muddy bottom; seagrass; sponges; seaweed) and signal parameters (minimum frequency, maximum frequency, minimum duration and maximum duration) to test both variables for statistical dependence ($p < 0.05$).

CHAPTER 3: SOUND PRODUCTION IN SEAHORSES

This chapter addresses the bioacoustics research objective 1, 2 and 3. The work here tested the following hypothesis: -

1) Feeding clicks and growls differ in their acoustic signals and sound producing mechanisms.
2) Feeding clicks are individual- and species-specific in seahorses.

3.1 Results

3.1.1 Time-frequency analysis

Two distinct sounds were recorded from all three species of seahorses (*Hippocampus comes, Hippocampus trimaculatus* and *Hippocampus barbouri*). These sounds are called feeding clicks (Figure 3.1) and distress growls (Figure 3.2). The seahorse only made one click per head movement whereas multiple distress growls could be recorded during the period the fish was held.

The oscillogram of all "feeding clicks" revealed an initial wave component (Figure 3.3a, A-B), followed by the acoustic energy burst of high frequencies (B-C) and another acoustic energy component of low frequency (C'-D). The energy spike of the high-frequency component decayed rapidly (C-C') within 5.0 ms with a time constant (τ) of 0.006± 0.002 s^{-1}, while the peak of the low-frequency component decayed more slowly, within 40 ms from D-E ($\tau = 0.028 \pm 0.014$ s^{-1}). The scalogram shows that the low-frequency and high-frequency energy bursts (red to yellow colour flares, Figure 3.3b) are coincident with the energy spectral density peaks (Figure 3.3c). The click sound with the high-frequency spike is however broadbanded with frequencies ranging from about 0.3 – 1.6 kHz in *H. comes*, 0.7 – 3.0 kHz in *H. barbouri* and 0.7 – 2.8 Hz in *H. trimaculatus*, while the low frequency sound is narrow banded within the first 300 Hz in all three species.

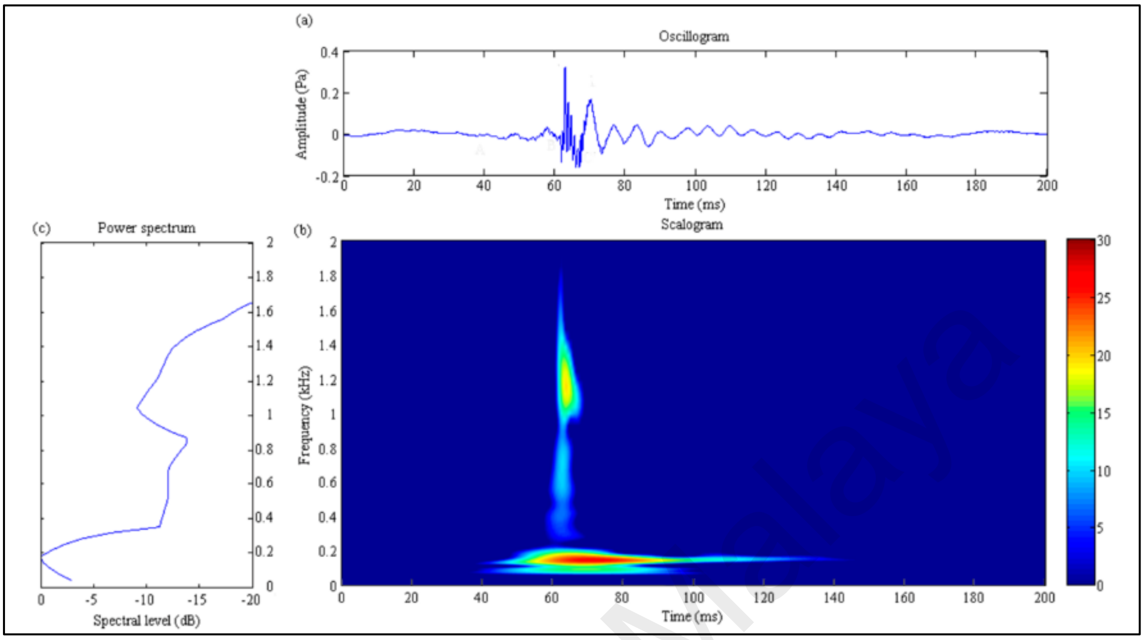

Figure 3.1: (a) Oscillogram, (b) scalogram and (c) energy spectral density of the feeding click produced by *Hippocampus comes*.

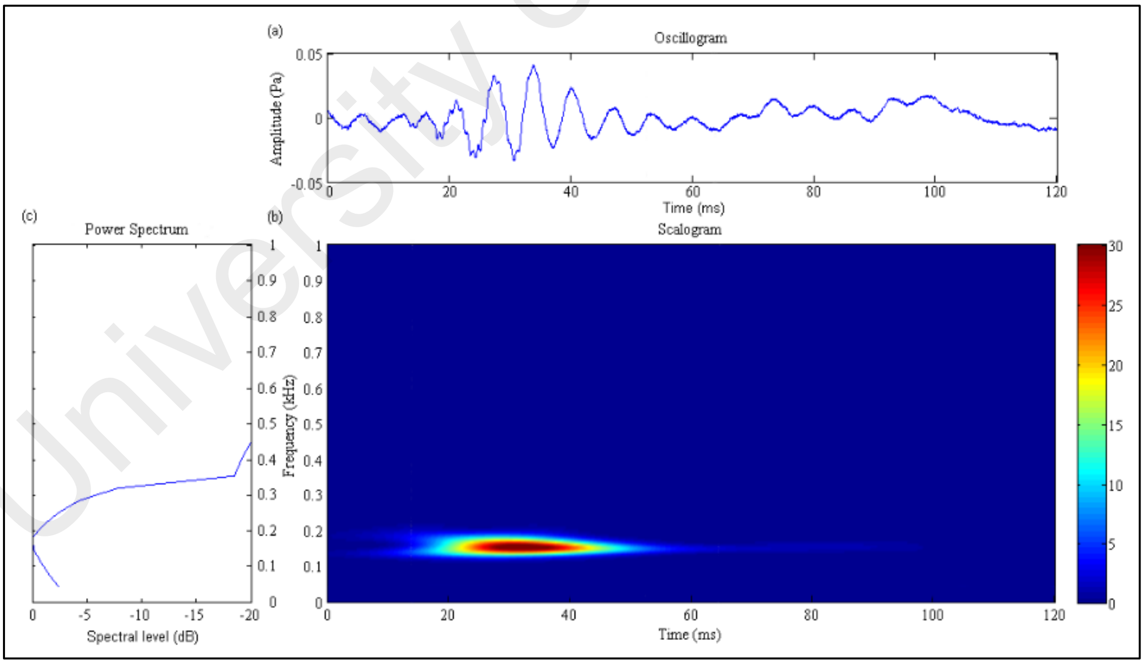

Figure 3.2: (a) Oscillogram, (b) scalogram and (c) energy spectral density of a distress growl produced by *Hippocampus comes*.

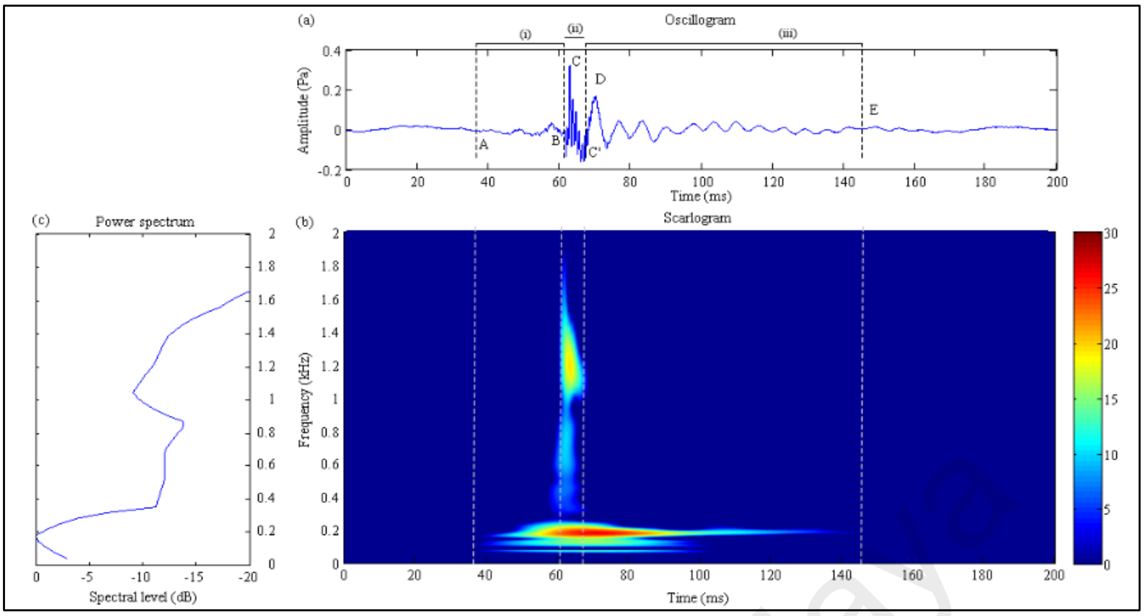

Figure 3.3: (a) Oscillogram, (b) scalogram and (c) energy spectral density of the feeding click produced by *Hippocampus comes*. (A-B) initial signal, (B-C) high-frequency signal, and (C-E) low-frequency sinusoidal signal and decay. Note the scalogram is time-matched to the oscillogram to show the measured durations of the three signals (i, ii, iii) respectively.

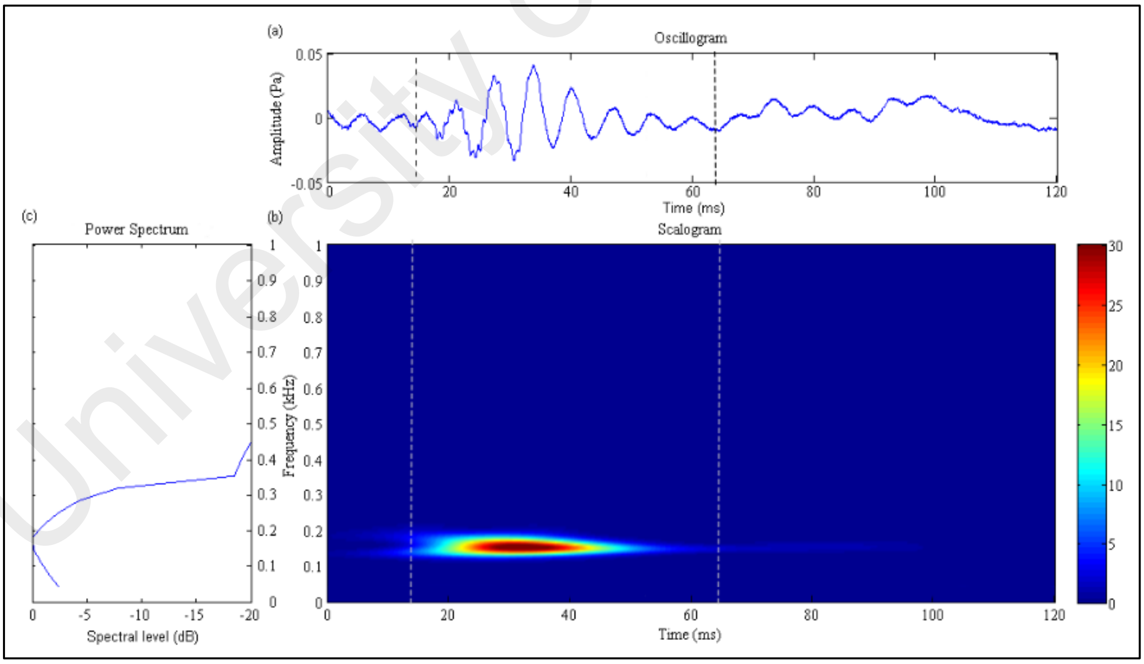

Figure 3.4: (a) Oscillogram, (b) scalogram and (c) energy spectral density of two distress growls produced by *Hippocampus comes*. Vertical lines in (a) and (b) indicate the measured signal duration.

In contrast to the feeding clicks, recorded growls from seahorses (Figure 3.4) displayed only a low-frequency component. The estimated time constant (τ) of the growl was 0.035 ± 0.021 s^{-1}.

3.1.1.1 *Hippocampus comes*

The initial wave signal of recorded "feeding click" in *Hippocampus comes* had a mean frequency of 0.16 ± 0.02 kHz (Table 3.1) and an increasing amplitude, with a duration of 7.8 ± 3.3 ms (Figure 3.3, A-B). Thereafter, the high- and low-frequencies of the "feeding click" had mean values of 1.19 ± 0.14 kHz, and 0.21 ± 0.07 kHz, and corresponding mean durations of 4.3 ± 1.2 ms (C-C') and 27.0 ± 8.8 ms (D-E), respectively. The high- and low-frequency components displayed significant differences in both their dominant frequency level (Paired t-test; $p < 0.05$) and duration (Paired t-test; $p < 0.05$) (Table 3.2).

Both high- and low-frequency components were consistently obtained in a total of 28 analyzed feeding clicks recorded from the six *H. comes*. Kruskal-Wallis non-parametric test indicated no significant differences in the click's dominant high frequency (Kruskal-Wallis test $H'_{5, 28} = 3.40$; $p > 0.05$) and duration ($H'_{5, 28} = 6.30$; $p > 0.05$) among individuals of the species (Table 3.2). For the low frequency component, there was also no significant difference among *H. comes* seahorses for its peak frequency ($H'_{5, 28} = 9.01$; $p > 0.05$). Its duration however shows significant difference ($H'_{5, 28} = 16.15$; $p < 0.05$), attributable to one seahorse which was the only female and bearing the shortest call duration. Removing this female from the ANOVA however gave no significant difference ($H'_{4, 16} = 4.40$; $p > 0.05$) in the duration among the five males (Table 3.2). The differences in the acoustic parameters suggest that the possibility that the high- and low-frequency components are products of different mechanisms (discussed later in the thesis).

Forty recorded growls from five *H. comes* seahorses (Figure 3.4) displayed only a low-frequency component which had a mean frequency of 0.17 ± 0.01 kHz and a mean duration of 46.3 ± 16.6 ms (Table 3.1). A minor reflection of the distress growl is clearly visible after the 65.0 ms mark in Figure 3.4 which was not taken into account when measuring the growl duration. The dominant frequency of the growl was significantly lower (Paired t-test; $p < 0.05$) than the high-frequency component in the click, but not significantly different (Paired t-test; $p > 0.05$) from the low-frequency component. The difference in duration between the click's high-frequency component and the growl's frequency was also significantly different (Paired t-test; $p < 0.05$). However, the duration of the low-frequency component of the click was significantly shorter than the growl's duration (Paired t-test; $p < 0.05$). The growl duration among *H. comes* was also not significantly different ($H'_{4,40} = 5.15$; $p > 0.05$).

3.1.1.2 *Hippocampus barbouri*

Only four feeding clicks were recorded from one of six individuals of *H. barbouri* due to animal in distress and notfeeding. All recorded clicks revealed a precursor signal with mean frequency of 0.49 ± 0.14 kHz and mean duration of 6.3 ± 0.9 ms followed by a spike as viewed on the waveform (Figure 3.5a). Similar to the recorded signals of *H. comes*, an energy burst was consistently observed at 1.39 ± 0.01 kHz with duration of 5.4 ± 1.67 ms (Table 3.1). Unlike that of *H. comes*, no low frequency component was respectively visible in the feeding clicks of *H. barbouri* but two energy concentrations were detected at 0.63 ± 0.15 Hz and at 2.70 ± 0.15 kHz with durations of 11.13 ± 2.45 ms and 4.82 ± 2.45, consistently in all 4 clicks. (Figure 3.5b) (Table 3.1). Due to low sample size and only one individual produced the feeding clicks, no analysis could be carried out on the intraspecific differences.

Seventy-four distress growls from six *H. barbouri* displayed low frequency narrow band energy at 0.17 $\pm$ 0.01 kHz with duration of 37.93 $\pm$ 5.99 ms (Figure 3.6) (Table 3.1). The distress growls among individuals of *H. barbouri* reveal intraspecific differences for its dominant frequencies ($H'_{5,\ 72} = 24.49$; $p < 0.05$) but no differences were detected for the duration of the growls ($H'_{5,\ 72} = 13.28$; $p > 0.05$) (Table 3.2). Further analysis using non-parametric equivalent of Tukey's test revealed that only one individual (HB04, male) to be different than the others. No differences between individuals of the species were detected after the removal of individual HB04 ($H'_{4,\ 59} = 11.47$; $p = 0.31$).

3.1.1.3 *Hippocampus trimaculatus*

A total of ten feeding clicks was recorded from six individuals of *H. trimaculatus.* Similar to the recorded waveforms of feeding clicks of *H. comes* and *H. barbouri*, a precursor signal with mean frequency of 0.16 $\pm$ 0.01 kHz and mean duration of 8.5 $\pm$ 0.8 ms was observed in all clicks (Figure 3.7a). A localised spectral energy concentration was recorded at 1.17 $\pm$ 0.12 kHz with duration of 6.12 $\pm$ 1.24 ms accompanied by higher frequency broadband residua energy (Figure 3.9). A narrow banded low frequency component was also detected in nine of ten recorded (90.0%) clicks with a frequency 0.17 $\pm$ 0.02 kHz with duration of 27.09 $\pm$ 2.17 ms (Table 3.1). Comparison of the sound parameters of feeding clicks between individuals of *H. trimaculatus* did not detect differences within the species for both the dominant frequencies of the main component ($H'_{3,\ 10} = 3.63$; $p > 0.05$) and low frequency component ($H'_{3,\ 9} = 2.35$; $p > 0.05$). The durations of the main component ($H'_{3,\ 10} = 7.45$; $p > 0.05$) and low frequency component ($H'_{3,\ 9} = 1.16$; $p > 0.05$) also displayed no significant differences (Table 3.2).

Table 3.1: Parameters of feeding clicks and distress growls of three species of seahorses, *Hippocampus comes*, *Hippocampus barbouri* and *Hippocampus trimaculatus*.

Parameters	Species		
	Hippocampus comes	*Hippocampus barbouri*	*Hippocampus trimaculatus*
No. of individuals	6	6	6
Mean standard height (cm)	11.6 ± 1.07	12.75 ± 0.81	11.58 ± 0.59
Occurrence of low frequency component (%)	100.0	100.0	90.0
Number of feeding clicks (n)	28	4	10
Mean frequency (kHz)			
Low frequency component	0.21 ± 0.07	0.63 ± 0.15	0.17 ± 0.02
Precursor frequency	0.16 ± 0.02	0.49 ± 0.14	0.16 ± 0.01
Dominant frequency	1.19 ± 0.14	1.39 ± 0.01	1.17 ± 0.12
Mean duration			
Low frequency	27.0 ± 8.8	11.1 ± 2.5	27.1 ± 2.2
Precursor frequency	7.8 ± 3.3	6.3 ± 0.9	8.5 ± 0.8
Dominant frequency	4.3 ± 1.2	5.4 ± 1.7	6.1 ± 1.2
Number of distress growls (n)	40	74	51
Mean frequency (kHz)	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.01
Mean duration (ms)	46.3 ± 16.6	37.93 ± 5.99	42.12 ± 7.53

Table 3.2: Summary of *p*-values and significance in the study of intraspecific differences in sound parameters of feeding clicks and growls of three species of seahorses, *Hippocampus comes, Hippocampus barbouri* and *Hippocampus trimaculatus*.

	p-values					
Parameters	df	*H. comes*	df	*H. barbouri*	df	*H. trimaculatus*
Intraspecific						
Feeding click	5				3	
Frequency						
Low frequency component		0.11*		na		0.50
Dominant frequency		0.64		na		0.30
Duration						
Low frequency component		0.01**		na		0.76
Dominant frequency		0.28		na		0.59
Distress growl	4		5		4	
Frequency		0.68		0.00**		0.07
Duration		0.27		0.68		0.00**

na = Insufficient replicates to perform analysis; * = significant; ** = highly significant
df = degree of freedom.

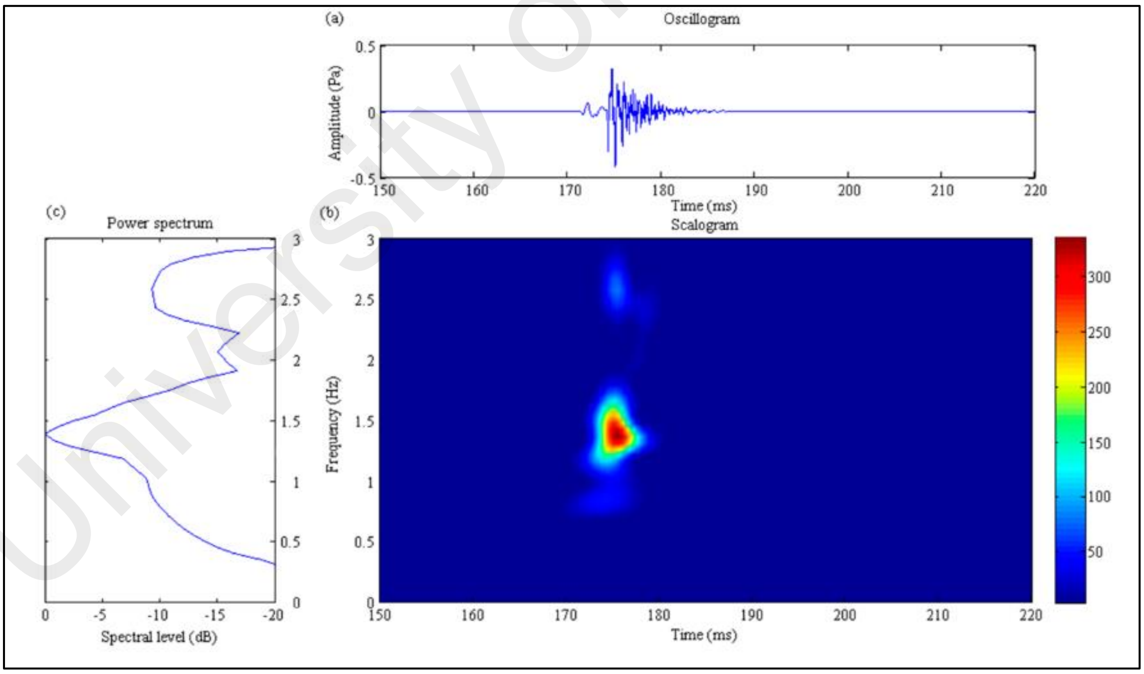

Figure 3.5: (a) Oscillogram, (b) scalogram and (c) energy spectral density of a feeding click produced by *Hippocampus barbouri.*

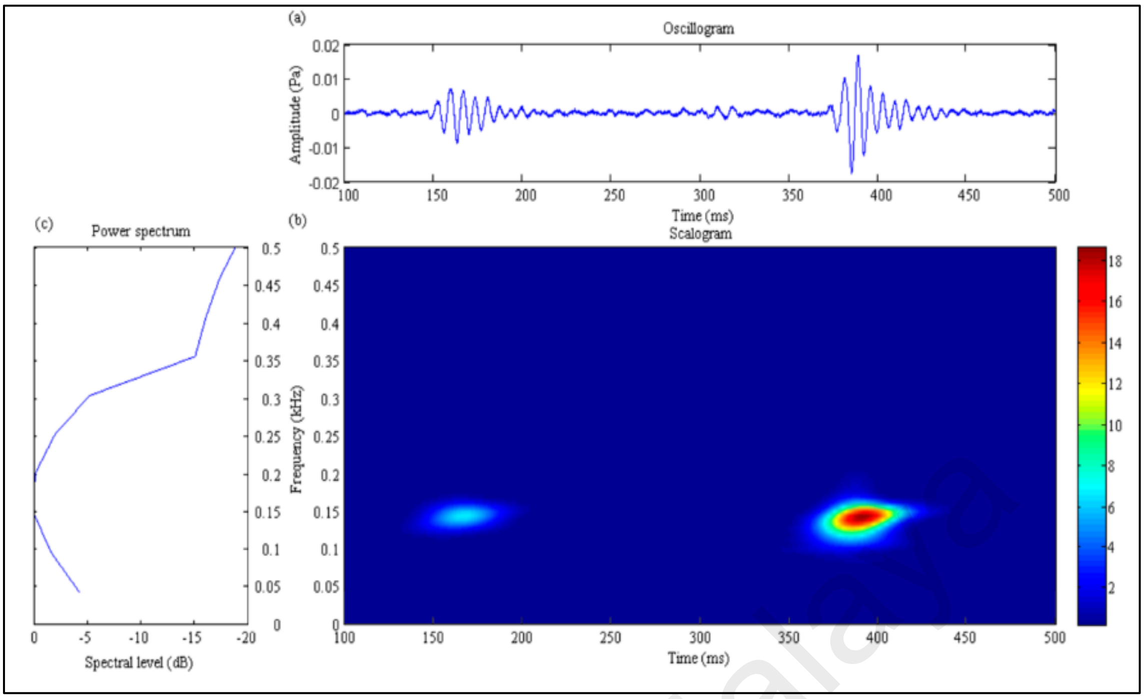

Figure 3.6: (a) Oscillogram, (b) scalogram and (c) energy spectral density of two distress growls produced by *Hippocampus barbouri*.

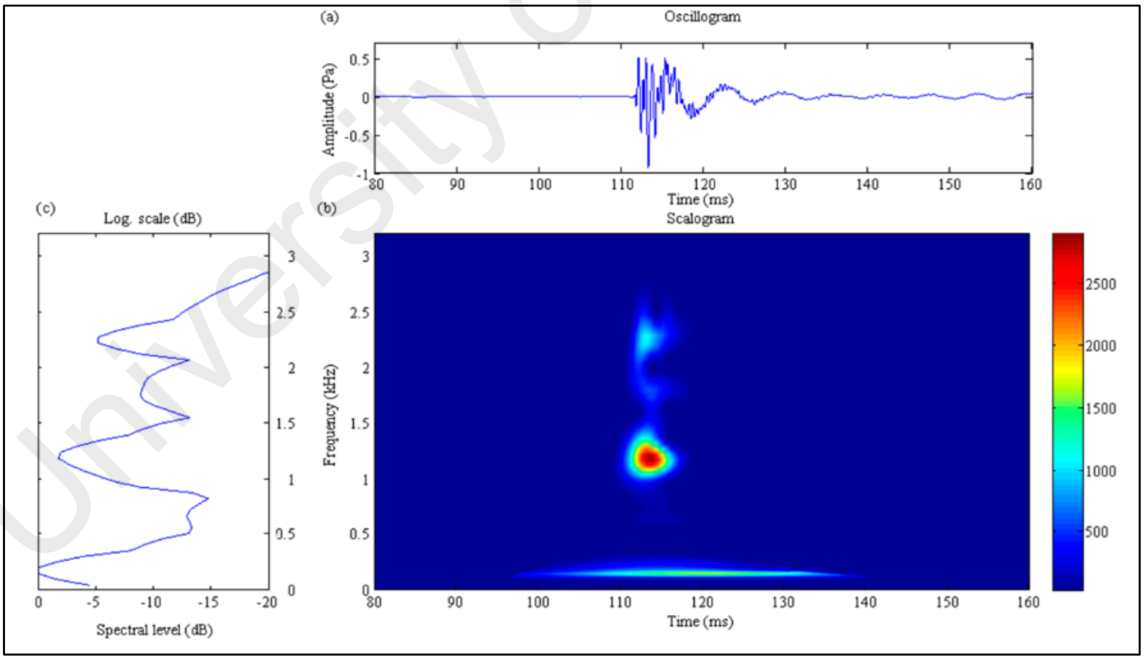

Figure 3.7: (a) Oscillogram, (b) scalogram and (c) energy spectral density of a feeding click produced by *Hippocampus trimaculatus*.

A total of 51 distress growls were recorded from five *H. trimaculatus* seahorses with a mean frequency of 0.16 ± 0.01 kHz with duration of 42.12 ± 7.53 ms (Figure 3.8) (Table 3.1). The waveform of growls is similar to *H. comes* and *H. barbouri* depicting a sinusoidal form (Figure 3.8). Comparison of distress growl frequencies between individuals of the species did not record any significant differences ($H'_{4, 52} = 8.77$; $p > 0.05$) (Table 3.2). However, differences were detected in the duration of growls between the individuals ($H'_{4, 52} = 16.50$; $p < 0.05$) (Table 3.2). Further analysis using non-parametric equivalent Tukey's test post-hoc test revealed only one individual to be significantly different than the others. There were no significant differences between the other four individuals of *H. trimaculatus* for growl duration once the significant individual was removed from analysis ($H'_{3, 40} = 4.80$; $p = 0.19$).

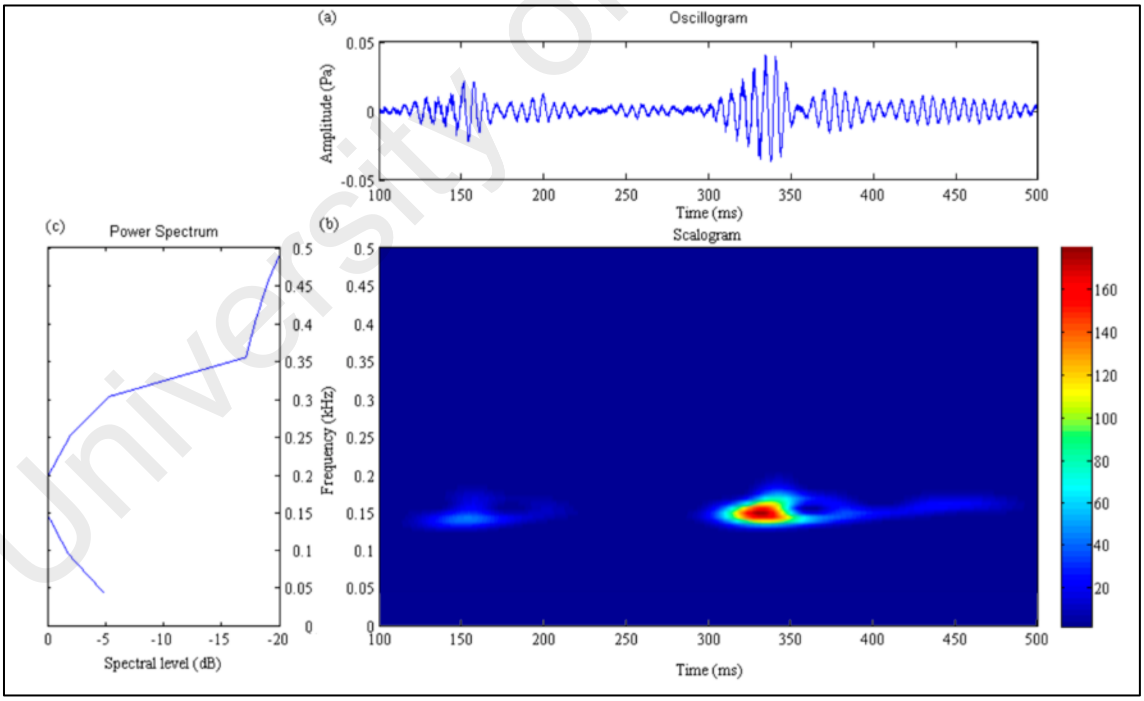

Figure 3.8: (a) Oscillogram, (b) scalogram and (c) energy spectral density of a distress growl produced by *Hippocampus trimaculatus.*

3.1.1.4 Comparison among species

Within feeding click signals, the low frequency component recorded among three seahorse species displayed interspecific differences ($p < 0.05$) for both frequency ($H'_{2, 41} = 27.31$) and duration ($H'_{2, 41} = 10.29$) parameters. Further analysis into the differences of feeding click components revealed frequency of the low frequency component *H. comes* to be very different ($p < 0.05$) with *H. barbouri* and *H. trimaculatus* but no differences ($p > 0.05$) were detected between *H. barbouri* and *H. trimaculatus*. Conversely, *H. barbouri* displayed significant differences ($p < 0.05$) from the other two seahorse species while no significant differences ($p > 0.05$) were detected between *H. comes* and *H. trimaculatus* (Table 3.3).

Similarly, the dominant frequency component was observed to display interspecific differences ($p < 0.05$) for frequency ($H'_{2, 42} = 29.04$) and duration ($H'_{2, 42} = 8.83$). It displayed a similar outcome to that of the low frequency component where *H. comes* was found to be very different ($p < 0.05$) from *H. barbouri* and *H. trimaculatus,* and there were no differences ($p > 0.05$) between *H. barbouri* and *H. trimaculatus* (Table 3.3). However, the duration of the dominant component displayed differences between *H. comes* and *H. trimaculatus* whereas *H. barbouri* is not significantly different ($p > 0.05$) from *H. comes* and *H. trimaculatus* (Table 3.3).

For the distress growls, the recorded frequency ($H'_{2, 164} = 92.28$) and duration ($H'_{2, 164} = 39.90$) parameters were also observed to display interspecific differences ($p < 0.05$) (Table 3.3). The Dunn's non-parametric comparison (Kruskal-Wallis post-hoc) test revealed *H. comes* to be significantly different from *H. barbouri* and *H. trimaculatus* for both frequency and duration of the distress growl ($p < 0.05$) whereas, *H. barbouri* and *H. trimaculatus* are not significantly different ($p > 0.05$) (Table 3.3). All recorded scalogram of feeding click depict a localised energy burst along with a broadband frequency spike.

A low frequency narrowband was also observed in feeding clicks across all three species. The depicted spectra forms of both the feeding clicks and distress growls are qualitatively similar among the three species.

Table 3.3: Summary of Kruskal-Wallis *p*-values and significance in the study of interspecific differences in sound parameters of feeding clicks and growls of three species of seahorses, *Hippocampus comes*, *Hippocampus barbouri* and *Hippocampus trimaculatus*.

		p-values				
Parameters	*df*	*H. comes*	*df*	*H. barbouri*	*df*	*H. trimaculatus*
Feeding click						
Frequency						
Low frequency component						
H. comes		-		0.04**		0.00**
H. barbouri		0.04**		-		1.00
H. trimaculatus		0.00**		1.00		-
Dominant frequency						
H. comes		-		0.03**		0.00**
H. barbouri		0.03**		-		1.00
H. trimaculatus		0.00**		1.00		-
Duration						
Low frequency component						
H. comes		-		0.00**		1.00
H. barbouri		0.00**		-		0.02**
H. trimaculatus		1.00		0.02**		-
Dominant frequency						
H. comes		-		0.58		0.01**
H. barbouri		0.58		-		1.00
H. trimaculatus		0.01*		1.00		-
Distress growl						
Frequency						
H. comes		-		0.00**		0.00**
H. barbouri		0.00*		-		0.61
H. trimaculatus		0.00*		0.61		-
Duration						
H. comes		-		0.00**		0.00**
H. barbouri		0.00**		-		0.14*
H. trimaculatus		0.00**		0.14*		-

* = significant; ** = highly significant;
df = degree of freedom.

3.1.1.5 Fast fourior transform (FFT)

Fast furior transform was applied to ony one species of seahorse to enable the comparison of this method with time frequency analysis. The spectral characteristics of the feeding click of *Hippocampus comes* using spectrogram revealed an energy peak over 2.5 kHz followed by rapid energy decay (Figure 3.9). The spectrogram shows a non-harmonic structure of the analysed feeding click sound. Additionally, a low frequency narrow band of between 0.1 – 0.2 kHz was clearly visible along with the energy peak. The power spectra (Figure 3.10) also revealed the two frequency peaks observed in the spectrogram.

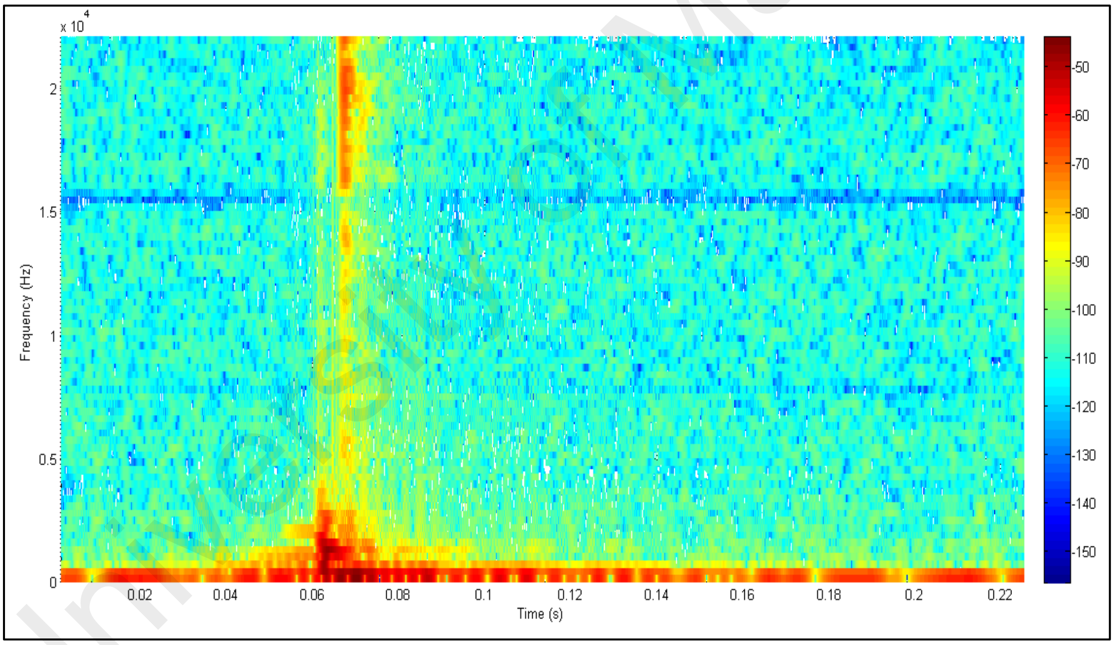

Figure 3.9: Spectrogram of feeding click produced by the tiger-tail seahorse, *Hippocampus comes*.

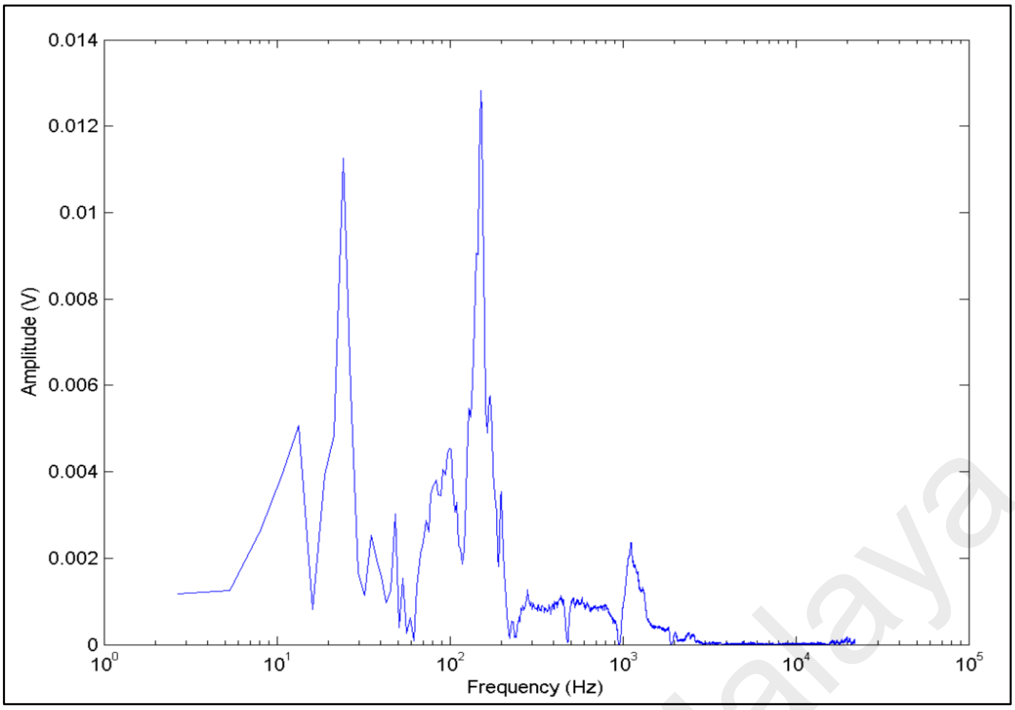

Figure 3.10: Amplitude spectra of feeding click produced by the tiger-tail seahorse, *Hippocampus comes*.

3.1.2 Head movements and morphology

At the time of click emission, the seahorse was observed to display a rapid elevation of the head with simultaneous depression of the hyoids. Using *H. comes* as a case study, the high-speed camera recordings estimated a mean duration of 645.0 ± 26.9 ms (*n* = 8) for *H. comes* to initiate and complete a successful feeding strike of the proffered prey (Figure 3.11). A feeding strike began from the resting stage (0.0 ms) to the quick striking stage in 6.0 ms before the head and body completely relaxed to their resting position after the strike in 626.0 ms.

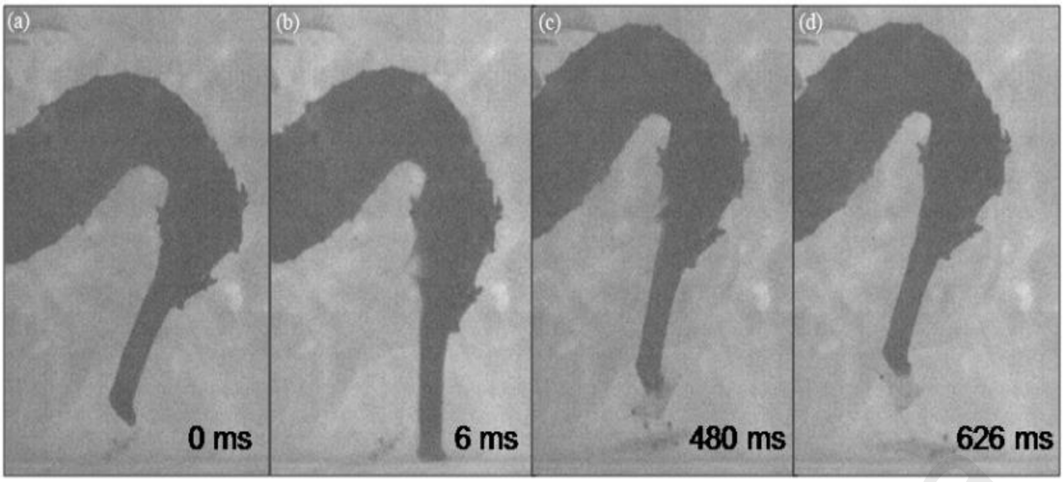

Figure 3.11: High-speed images of *Hippocampus comes* feeding strike: - (a) Seahorse feeding strikes begins from the resting stage, (b) before simultaneously striking its prey with the depression of its hyoid and, (c-d) returns to the resting stage completely.

3.1.2.1 *Hippocampus comes*

All clicks (n= 28) produced were observed to be associated with head movement and the expansion of the hyoid, while no similar movements were observed (n = 40) with the production of growls.

Microtomographic scans of the head of *H. comes* reveal the unique articulation of the coronet (COR) bone with the supraoccipital (SOC) bone on the dorsal aspect of the cranium (Figure 3.12a). Underneath the skin fold of the head, the ridge or 'beak' of the supraoccipatal bone articulates with the posterior keeled coronet bone (Figure 3.12b). There is no true articulation, as by tendon or muscle, and the two bone pieces are free from each other. However, when the head is flexed backwards, the beak of the supraoccipital bone is brought down, fitting perfectly with the anterior keel of the coronet. The matching surface contours of the SOC beak and the COR keel is evident in the microtomograph. Also, the microtomograph clearly shows a tissue-free space below these two bones. This space was apparently fluid-filled for it dried out leaving a space in the dried specimen. The tip of the crown in *H. comes* features a low coronet with several distinct knobs.

3.1.3.2 *Hippocampus barbouri*

Feeding clicks (n= 4) were associated with head movement and the expansion of the hyoid while no similar movements were observed (n = 74) with the production of growls. Alizarin stained COR and SOC bones of *H. barbouri* reveal a similar articulation as that of *H. comes* with matching surface contours and independent from one another (Figure 3.13). A tissue free space was observed under the COR and SOC bones. The coronet crown of *H. barbouri* features a high extension with several spines.

3.1.3.3 *Hippocampus trimaculatus*

Similar to *H. comes* and *H. barbouri*, head movement and hyoid expnsion were associated with all feeding clicks (n=10) while no similar movements were observed with growls (n=51). Reconstructed microtomography images of the SOC and COR bones of *Hippocampus trimaculatus* also shows the empty space underneath both bones (Figure 3.14a). Similarly, matching surface contours suggest that when the head of the seahorse moves backwards, the SOC beak would slide and fill the empty space (Figure 3.14b). Both the SOC and COR show no true articulation and are independent from one another. The *H. trimaculatus* features a low coronet that looks similar to that of *H. comes* but both are different from the coronet shape of *H. barbouri*.

3.1.3.4 Other seahorse species

No video and sound recording were made for *Hippocampus spinosissimus* and *Hippocampus kuda* due to unavailability of live specimens. Therefore, head movement and hyoid expansion could not be observed. However, microtomographic reconstructed and stained SOC and COR regions of both the *H. spinosissimus* (Figure 3.15a, b) and *H. kuda* (Figure 3.16) revealed similarities in shape and orientation as the other previous species of seahorses (*H. comes*, *H. trimaculatus* and *H. barbouri*). The space between both the SOC and COR bones were present (Figure 3.15a) and both bones are observed to be independent of one another and covered by a layer of skin (Figure 3.15b and 3.16). The SOC slides on the COR and occupies the empty space when the head of the seahorse flexes backwards. The coronet of *H. kuda* generally protrudes backwards and is rounded in nature which is in contrast to *H. spinosissimus* which possess a high coronet with four to five spines.

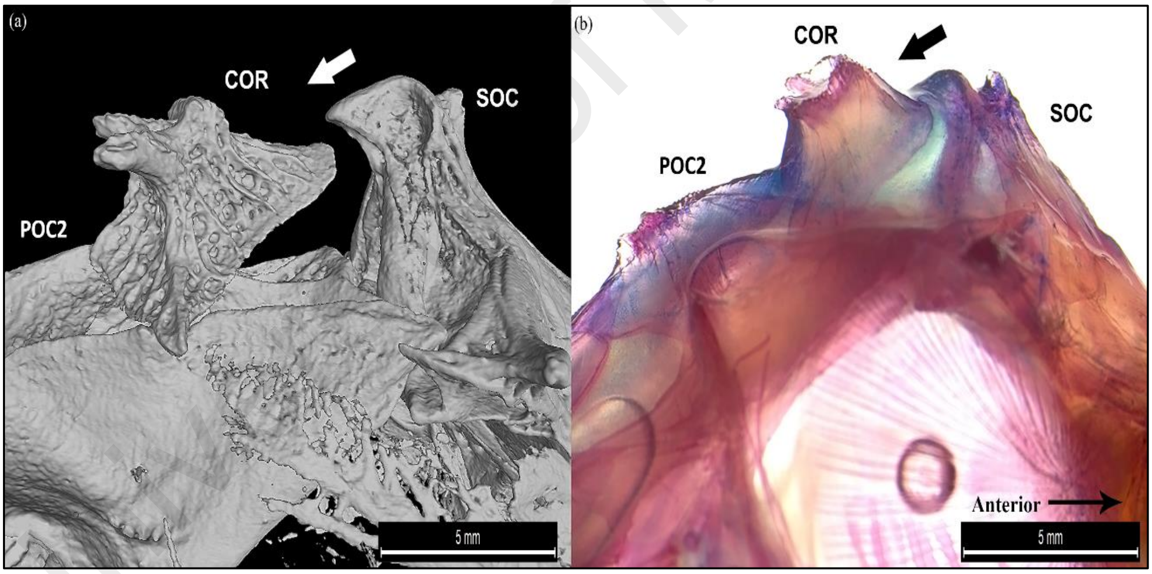

Figure 3.12: (a) Reconstructed tomography image and (b) Alizarin Red stained coronet (COR), postcranial plate 2 (POC2) and supraoccipital (SOC) region of *Hippocampus comes* facing the right. Arrow indicates the direction of sliding movement of SOC bone producing the click.

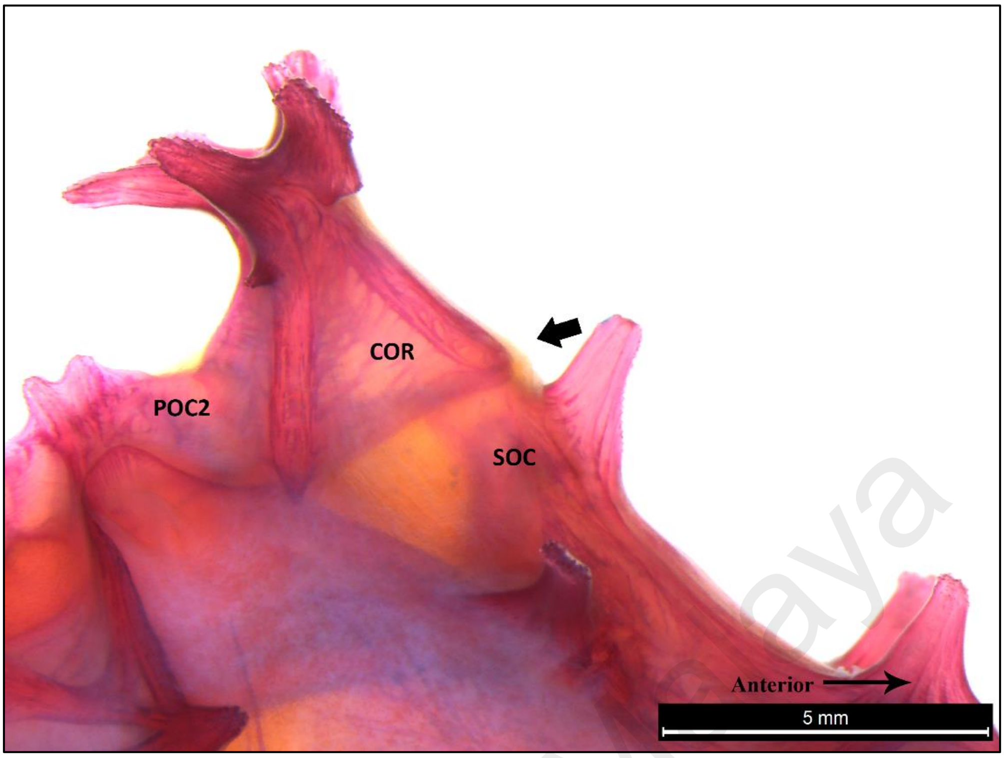

Figure 3.13: Alizarin Red stained coronet (COR), postcranial plate 2 (POC2) and supraoccipital (SOC) region of *Hippocampus barbouri* facing the right. Arrow indicates the direction of sliding movement of SOC bone producing the click.

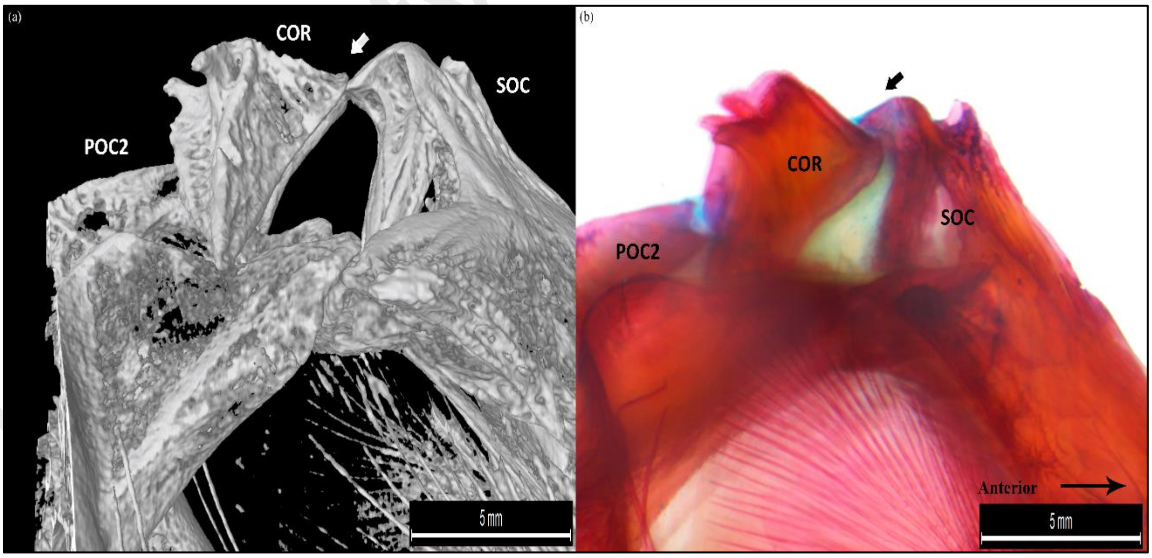

Figure 3.14: (a) Reconstructed tomography image and (b) Alizarin Red stained coronet (COR), postcranial plate 2 (POC2) and supraoccipital (SOC) region of *Hippocampus trimaculatus* facing the right. Arrow indicates the direction of sliding movement of SOC bone producing the click.

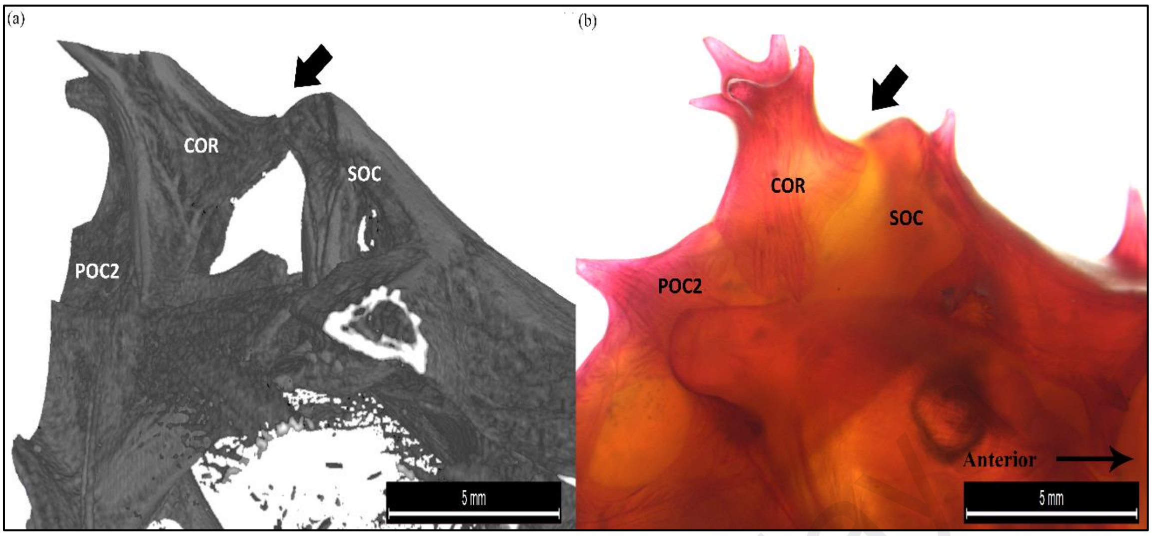

Figure 3.15: (a) Reconstructed tomography image and (b) Alizarin Red stained coronet (COR), postcranial plate 2 (POC2) and supraoccipital (SOC) region of *Hippocampus spinosissimus* facing the right. Arrow indicates the direction of sliding movement of SOC bone producing the click.

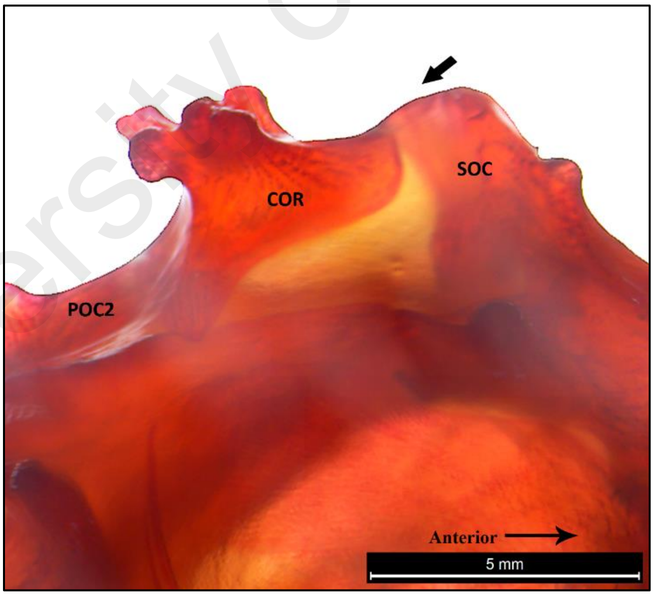

Figure 3.16: Alizarin Red stained coronet (COR), postcranial plate 2 (POC2) and supraoccipital (SOC) region of *Hippocampus kuda* facing the right. Arrow indicates the direction of sliding movement of SOC bone producing the click.

3.1.3 Growl vibrations in seahorses

Vibration recordings at various points along the body of *Hippocampus comes* were done to locate the origin of the low-frequency growls since the origin will transmit the strongest vibration. Point B on the cheek of the fish (Figure 3.17) displayed the highest mean acceleration amplitude of 0.97g, whereas regions away from the cheek displayed increasingly dampened values the farther they were away from the cheek. Only the tip of the snout showed a higher value than expected from its distance to the cheek. The mean vibration amplitude (0.25g) at point E or nearest to the swim bladder was nearly four orders of magnitude lower than at the cheek. The dominant frequency values of vibration recorded at all points fell within the range of 150 – 200 Hz.

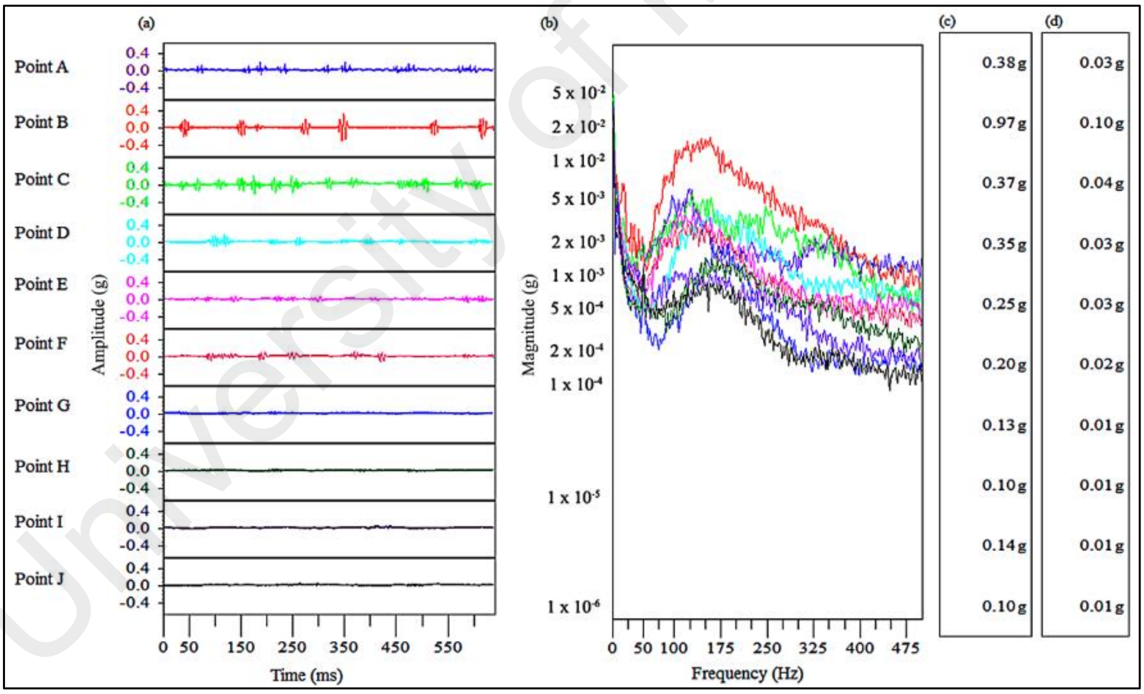

Figure 3.17: (a) Oscillogram of vibrations recorded at ten points (A-J) on the body of *Hippocampus comes*, (b) fast fourier transform of recorded data to detect peak amplitude (log scale) and vibration frequency, (c) computed mean maximum amplitude at body points, and (d) standard deviation of maximum amplitude. See Figure 2.2 for detailed explanation of body points.

3.2 Discussion

Feeding click recordings of the tiger tail seahorse, *Hippocampus comes*, were analysed using two different approaches: 1) fourier transformation and 2) Morlet wavelet transformation. The spectrogram of the feeding click of *H. comes* generated by fast fourier transformation was found to be heavily pixelated due to its limited resolution compared to the scalogram generated by the wavelet transformation approach. The Fourier transformation utilises a fixed window which is limited by its resolution capability in that the selected window remains fixed for all time and frequency parameters (Flandrin *et al*. 1991). In contrast, the scalogram simultaneously provide an optimal time and frequency resolution by using superior analytical capability to study highly irregular signals. For example, the oscillogram, power spectrum and scalogram of the analysed signal were time and frequency matched in contrast to the single plane (time or frequency) window of the conventional approach. Since the time-scale analysis is logarithmic in frequency, the area of influence of some pure frequency *fo* in the signal increases with *fo* in the scalogram; therefore, the scalogram depicts 'instantaneous spectral content' (Rioul and Vetterli, 1991).

The wavelets of the feeding click (Figure 3.1, scalogram) give smoothed energy bands because they include both real and imaginary parts of the signal, in comparison to the spectrogram output of pixelated energy bands (Figure 3.9). Better readability is provided by plotting only the absolute values which reduces the noise components (Rioul and Vetterli, 1991; Fedotenkova and Hutt, 2014). The wavelet transformation approach is suitable for studying signals with singularities and noises since it averages the intensity of the available components by removing noise in the process. The fourier transformation approach however, is affected by the available noise signals in any recordings, which appear along with the generated signal energies.

The sound parameters of both the fast fourier transformation (Figure 3.9) and the wavelet transformation (Figure 3.1) of *Hippocampus comes* feeding clicks yield similar values. Hence, the latter or non-conventional method does not give any distortion and misinterpretation of the analysed sound values. However, due to its better resolution, noise reduction and signals matched on both the time and frequency scales, the wavelet transformation method allows better interpretation, and potentially provides better insight in fish bioacoustical studies.

The signal waveform of all recorded seahorse feeding clicks precedes with an initial signal (Figure 3.5; 3.7; 3.9) that was also observed in previous studies (Fish, 1953; Colson *et al*., 1998; Anderson, 2009; Chakraborty *et al*., 2014; Oliveira *et al*., 2014). However, these studies made no mention of this signal. Similar initial signals have also been documented in snapping shrimps, *Alpheus heterochaelis* and *Synalpheus paraneomeris* (Au and Banks, 1998; Versluis *et al*., 2000).

Reconstructed microtomographic and histological images of five seahorse species reveal that the orientation and morphology of the sound producing mechanism to be morphologically similar. The viewings suggest that the supraoccipital (SOC) beak would make contact against the coronet (COR) keel when the head is flexed backwards when it is pulled by the epaxial tendon (and result in the production of broadband frequency spike. As the SOC slides backwards (giving the precursor signal), it also depresses the wedge-shaped COR bone (much like cocking a revolver hammer) which recoils to forcefully strike the SOC bone. Inevitably, such a mechanism would generate frictional force and heat, which presumably are dissipated in the fluid-filled space. This explains why the high-frequency component decayed very rapidly, while the subsequent low-frequency sinusoidal signal that originates elsewhere may decay more slowly.

The feeding click spectral forms (scalogram) of three species of seahorses, *H. comes* (Figure 3.6), *H. babouri* (Figure 3.7) and *H. trimaculatus* (Figure 3.9) are qualitatively similar to one another and with that of previous studies, depicting an initial burst of energy followed by a rapid decay of the signal energy (Colson *et al*., 1998; Anderson, 2009; Chakraborty *et al*., 2014; Haris *et* al., 2014; Oliveira *et al*., 2014). The duration of click sounds of the seahorses are also similar to that of *H. zosterae* and *H. reidi* which have an average click duration of 5 - 20 ms (Colson *et al*. 1998) and 16.1 ms (Oliveira *et al.,* 2014) respectively. The range of click peak frequencies for *H. comes* (1.1 kHz to 2.8 kHz), *H. trimaculatus* (1.0 kHz to 2.4 kHz) and *H. barbouri* (1.4 kHz to 3.0 kHz), overlapped with those of *H. erectus* (2.0 kHz to 2.4 kHz) (Colson *et al*., 1998) and *H. zosterae* (2.7 kHz to 3.4 kHz). The clicks of seahorses also displayed broadband frequency spectra that ranged from tens of hertz to <3 kHz, comparable with observations made by Colson *et al*. (1998), Oliveira *et al*. (2014) and Chakraborty *et al*. (2014). It is possible that different seahorse species produce specific acoustic signals due to morphological differences (shape and size) in their sound-producing bones. Other factors such as fish size and sex may also affect sound parameters. For instance, a negative correlation between sound peak frequency and fish size has been reported in *H. zosterae* (Colson *et al*., 1998), whereas in *H. reidi*, no differences in acoustic parameters between sex were reported (Oliveira *et al*. 2014).

The final low-frequency sinusoidal component, as revealed by wavelet analysis, is a new component in the seahorse "click" and has never been reported before. Previous researchers failed to detect this component because they used methods that only revealed the high-frequency component but did not detect this low-frequency component (see Fish, 1953; Colson *et al*., 1998; Chakraborty *et al*., 2014; Haris *et al*., 2014). Our study further reveals that the temporal patterns of the low-frequency and high-frequency components are different. This suggests that the seahorse “click” is a compounded sound that likely results from a dual sound producing mechanism. As our study shows, a similar low-frequency growling sound can also be produced by the seahorse under duress. This growl has no precursor signal like in the click. Both the growl and the final low-frequency signal in the “click” are however similar in terms of spectral characteristics.

Unlike the feeding click which is accompanied by head movement and depression of the hyoid, none of these movements were observed for the growl. This further supports the notion that the growl is produced by a different mechanism and independent of the click mechanism. However, it is tempting to suggest that both the growl and the low-frequency sinusoidal component of the “click” are produced by the same cheek mechanism since their time constants (τ) are close to each other, i.e. pointing to similar damping material. Nevertheless, the cheek mechanism that likely produces these two sounds appears to be triggered differently and under different conditions. The growl is triggered under duress; it could be repeated over a longer duration, while the low-frequency component of the “click” is one-off and of a shorter duration. Obviously, the click’s low-frequency component is not produced under duress, but in fact, produced possibly in a gratifying situation. Hence, this sound is distinguished as the purr.

The first trigger may be a by-product of the energy expended during the click’s high-frequency spike, which radiates from the SOC-COR area on the cranium to the bones at the cheek region. Since it is a broadband frequency spike (Figure 3.5, 3.7, 3.9), one may conjecture that the purr results from an effect similar to stochastic resonance (Gammaitoni *et al*.,1998; McDonnell and Abbott, 2009). In stochastic resonance, some oscillatory systems with specific natural frequency f_o can be excited by noise or input with broad spectral harmonic mixtures which include f_o. An example of how stochastic resonance triggered in fish could help it detect weak electrical noise generated by swarming zooplankton prey (Daphnia) has been reported in the juvenile paddlefish *Polyodon spathula* (Freund *et al*.,2002). Stochastic resonance occurring and triggering the cheek mechanism is supported by the observation that the purr, a one-off signal always occurs immediately (1–2 ms) after the high-frequency spike, even if the feeding strike of the seahorse was unsuccessful.

A second possible trigger of the purr could result spontaneously along with head elevation and hyoid depression during feeding. Here, like the growl, the purr is a self-generated mechanism which possibly involves the vibrations of certain "cheek" bones such as opercula, girdle bones, etc., that are located here. Further work is necessary to elucidate the cheek sound-producing mechanism of both the growl and purr. Nevertheless, one possibility is the vibrations of the pectoral-pelvic girdle described in the sculpin which lacks a swim bladder (Barber and Mowbray, 1956; Colleye *et al*., 2013). The wave form and low frequency signal of the seahorse growl in the present study match those of the sculpin. The pectoral girdle which is joined to the back of the cranium is located very close to the operculum of the seahorse (Leysen *et al*., 2011). Interestingly, a similar situation of radiating vibrations which are not from the cranium but from the buccal jaws occur in the clownfish *Amphiprion clarkia*; here, the jaw vibrations are propagated via the skeleton to the rib cage whose oscillations drum the swim bladder (Colleye *et al*., 2012). In the blue cat- fish, the pectoral spine rubs against the cleithrum eventually exciting the pectoral girdle into resonance (Mohajer *et al*., 2015). Thus, it appears that the slip-stick mechanism and the rub-and-knock mechanism are similar in terms of causing the pectoral girdle to vibrate producing the low-frequency sound (purr of the seahorse); in the former, by the pectoral spine and in the latter, by the SOC-COR bones. How this is achieved may differ due to spatial differences; the former by constructive reinforcement (the pectoral spine directly contacting the pelvic girdle)

(Mohajer *et al*., 2015), and the latter by stochastic resonance (the SOC-COR bones in indirect contact with the pelvic girdle).

Additionally, the reconstructed microtomographic and histiological images further revealed variations in the morphological structure of the COR bone and its height for each of the five seahorse species while the shape SOC bone is similar among the seahorse species. The coronet shapes, number of spines, height and positions are different in different seahorse species (Lourie *et al*., 2004). Since the feeding click is produced during the contact of the SOC on the COR bone, it can be hypothesised that the COR bone could potentially be responsible for the production of temporally varied signals in seahorses. Similar occurrence has been observed in different mailed catfish species whereby the crests are distributed on the lateral surface of the dorsal protrusion of the ray, and their numbers, sizes, and shapes are different (Kaatz and Stewart, 1997). The appearance of a distinct pulse in the sound is likely to be generated during the rubbing of each crest upon a rough point (Fine *et al*., 1996; 1999).

In the present study, the propagation of sound vibrations from the coronet to the swim bladder in the seahorses has been considered. However, given the vibrational test results, this mode of propagation appears to be remote. Moreover, the swim bladder of all five species did not possess swim bladder muscles as also reported for *H. reidi* by Oliveira and her team (2014). Although swim bladder resonance has been experimentally demonstrated in the red-fin loach (Raffinger and Ladich, 2009) and the yellowfin tuna (Finneran *et al*., 2000), it seems unlikely that the seahorses' swim bladder can vibrate or resonate with the same energy that produces the growling or purring sound.

In summary, the feeding click of the seahorses displays three unique spectral signatures comprising of a low-frequency component, a high-frequency component and a low-frequency sinusoidal component whereas distress growl consists of a solitary low frequency component. The above supports the hypothesis of differing acoustic signals between feeding click and distress growl of the seahorses. The first two components result from the sliding movement and forceful contact between the COR and SOC bone, while the third component (purr) is generated elsewhere. Microtomographic and histological images of the COR and SOC region responsible for the production of clicks in five species of seahorses were all of similar orientation and shape. The growl of seahorses was also detected to originate from the cheek region when the seahorse is under duress. This finding confirms the hypothesis of different origins and sound producing mechanisms of feeding click and growl in seahorses. The spectral shapes of the feeding clicks and distress growls are the same for the three species of seahorses. However, sound parameters revealed no intraspecific variation within all three species whereas interspecific variation occurs among species for both sounds. Thus, the finding did not agree with the hypothesis of individual specific feeding click signal in seahorses while on the contrary, the results agree with the hypothesis of species specific signals. Further work is necessary to elucidate the cheek sound-producing mechanism of both the growl and purr.

CHAPTER 4: SOUND PRODUCTION IN PIPEFISHES

This chapter addresses the bioacoustics research objective 1, 2 and 3. The work here tested the following hypothesis: -

1) The click sound producing mechanism differs among species.
2) Feeding clicks are individual-specific and species-specific in pipefishes.

4.1 Results

4.1.1 Time-frequency analysis

All three species of pipefishes (*Doryichthys martensii, Doryichthys deokhatoides* and *Syngnathoides biaculeastus*) produced high frequency, short broadband clicks during feeding strikes. One click per head movement was observed consistently in all recorded clicks in all three species.

4.1.1.1 *Doryichthys martensii*

The feeding click waveform of *D. martensii* consists of an asymmetrical, single pulsed burst at 0.54 ± 0.03 kHz which decays rapidly (10.37 ± 1.10 ms) (Figure 4.1A, B). However, its scalogram also revealed a lower energy burst at higher dominant frequency of about 1.07 ± 0.04 kHz but with a shorter duration of 6.56 ± 2.47 ms (Figure 4.1 B) (Table 4.1). The power spectrum revealed an additional low frequency component of 0.17 ± 0.01 kHz, with duration of 26.05 ± 7.02 ms (Figure 4.1C, D) which can also be observed from the sine wave in the oscillogram (23.0-30.0 ms) and tonal frequency in the scalogram. This component was however observed in 77.8% of the total recorded signals (Table 4.1). The dominant frequency (Kruskal-Wallis test statistic, $H'_{df(3), N(18)} = 4.65$) and duration ($H'_{3, 18} = 2.76$) of all recorded clicks were not significantly different ($p > 0.05$) among four individuals within species. The low frequency component also did not record any significant differences ($p > 0.05$) in its sound characteristics in terms of frequency ($H'_{2, 19} = 1.30$) and duration ($H'_{2, 19} = 4.42$) within species (Table 4.1).

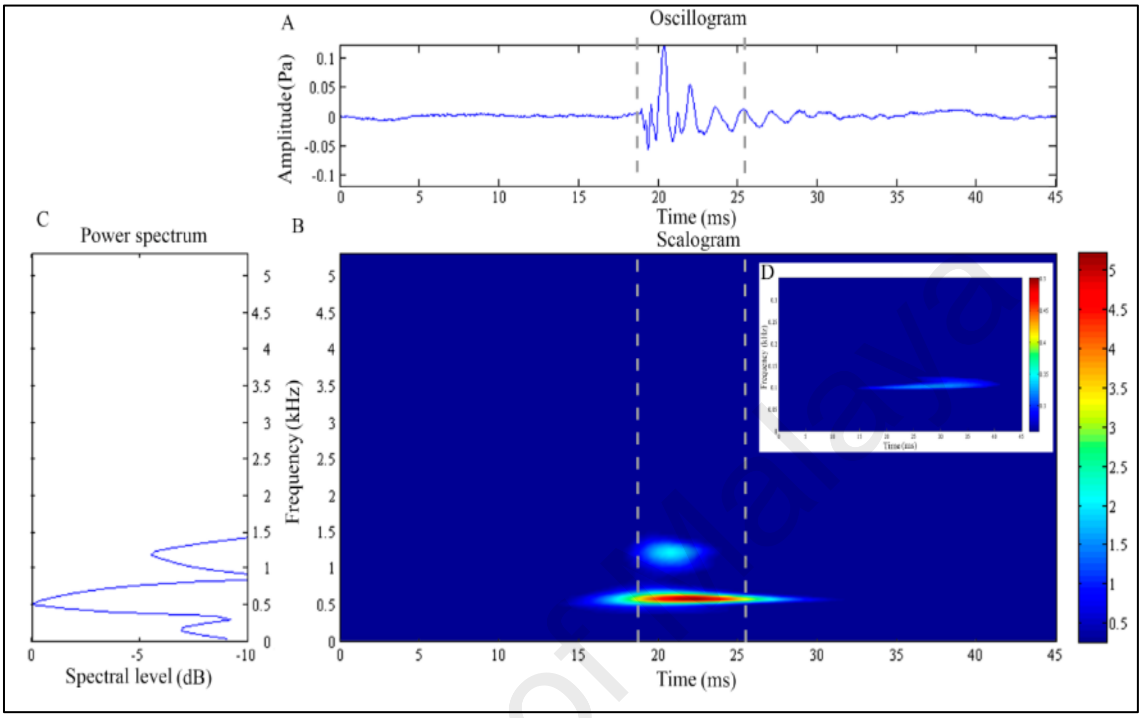

Figure 4.1: (A) Oscillogram, (B) scalogram (C) energy spectral density and (D) low frequency component of the feeding clicks produced by *Doryichthys martensii*. Vertical lines in (A) and (B) indicate the measured signal duration.

4.1.1.2 *Doryichthys deokhatoides*

The waveform of *D. deokhatoides* revealed symmetrical low-amplitude wave before and after a high-amplitude spike (Figure 4.2C). The scalogram revealed a broad band, high-frequency modulation energy burst of between 1.5 to 4.5 kHz which was of short duration (4.01± 1.18 ms) (Figure 4.2B) (Table 4.1). This broad band signal appeared to be the merging of two components, 2.04 ± 0.3 and 3.84 ± 0.16 kHz with dominant or modal frequencies of 2.55 ± 0.15 kHz (Figure 4.2C). A low frequency component was recorded at 0.20 + 0.03 kHz with an average duration of 10.35 ± 6.40 ms (Figure 4.2A, B). However, this low frequency component was observed in only 29.2% of the total recorded clicks for the species (Table 4.1). Both dominant frequency ($H'_{2, 14} = 5.97$) and duration ($H'_{2, 14} = 3.94$) of recorded feeding clicks of *D. deokhatoides* were not significantly different ($p > 0.05$) among the three individuals. Similarly, the low frequency component displayed no significant difference ($p > 0.05$) for frequency ($H'_{2, 13} = 0.70$) and duration ($H'_{2, 13} = 0.36$) within species.

4.1.1.3 *Syngnathoides biaculeatus*

The feeding click of *S. biaculeatus* displays a multimodal sinusoidal waveform of higher amplitude (Figure 4.3A) in contrast to the feeding clicks of both *Doryichthys* species. The waveform displays initial small precursor components, followed by a gradual build-up of large wave components in two or more overlapping pulses before they followed an extended decay. The double-pulse comprised an initial higher-amplitude (0.2 Pa) pulse of 4 ms, followed very quickly by a second lower-amplitude (0.1 Pa) pulse of 5 ms. The scalogram of *S. biaculeatus* displayed a localised energy burst at a dominant frequency of 2.09 ± 0.06 kHz with an average duration of 3.40 ± 0.75 ms (Figure 4.3B), followed by repeated broadband energy releases in the frequency range of 1.8 kHz to 6.6 kHz and over a duration of 8.64 ± 0.61 ms (Figure 4.3C) (Table 4.1). Only one recorded click shows an additional low-frequency component which was detected with a dominant frequency of 0.82 kHz and duration of 11.79 ms (not shown in figure). The dominant frequency ($H'_{5, 43} = 2.08$) and duration ($H'_{5, 43} = 2.52$) of all recorded clicks of *S. biaculeatus* was not significantly different ($p > 0.05$) among the six individuals of the same species.

Table 4.1: Feeding click parameters of three species of pipefishes, *Doryichthys martensii*, *Doryichthys deokhatoides* and *Syngnathoides biaculeatus*.

Characteristics

Individuals	Dominant Frequency		Low Frequency		High Frequency 1		Other Components	
	Mean Frequency (kHz)	Mean Duration (ms)	Mean Frequency (kHz)	Mean Duration (ms)	Mean Frequency (kHz)	Mean Duration (ms)	Mean Frequency (kHz)	Mean Duration (ms)
D. martensii								
DM01	0.58^{a}	9.7^{a}	0.17^{a}	31.7^{a}	1.08	4.67	-	-
DM02	0.54	9.2	0.17	28.3	1.12	4.26	-	-
DM03	0.51	11.0	0.17	18.2	1.06	9.25	-	-
DM04	0.51	11.6	-	-	1.03	8.05	-	-
Mean (±SD)	0.54 ± 0.03^{A}	10.37 ± 1.10^{A}	0.17 ± 0.01^{A}	26.05 ± 7.02^{A}	1.07 ± 0.04	6.56 ± 2.47	-	-
D. deokhatoides								
DD01	2.61^{b}	2.35^{b}	0.20^{b}	16.9^{b}	3.68	1.78	1.83	3.6
DD02	2.66	3.46	0.15	9.4	3.85	2.58	2.39	5.3
DD03	2.38	3.09	0.25	4.17	4.00	2.47	1.91	3.1
Mean (±SD)	2.55 ± 0.15^{B}	2.97 ± 0.57^{B}	0.20 ± 0.03^{A}	10.35 ± 6.40^{B}	3.84 ± 0.16	2.28 ± 0.43	2.04 ± 0.30	4.01 ± 1.18
S. biaculeatus								
SB01	2.09^{c}	3.1^{c}	-	-	-	-	-	-
SB02	2.14	2.8	-	-	-	-	-	-
SB03	2.08	3.1	-	-	-	-	-	-
SB04	2.05	4.1	0.82	11.8	-	-	-	-
SB05	2.17	4.5	-	-	-	-	-	-
SB06	2.03	2.7	-	-	-	-	-	-
Mean (±SD)	2.09 ± 0.06^{C}	3.40 ± 0.75^{B}	0.82 ± 0.01	11.79 ± 0.01	-	-	-	-

$^{a, b, c}$ All intraspecific differences are not significant.

$^{A, B, C}$ Indicate significant difference among species if different alphabets (alpha = 0.05).

4.1.1.4 Comparing among pipefish species.

Statistical analysis revealed interspecies differences for both click frequency and duration (Table 4.1). *Syngnathoides biaculeatus* was significantly different ($H'_{2, 94}$ = 54.35) ($p < 0.05$) from *D. martensii* and *D. deokhatoides* in dominant frequency but only *D. martensii* for duration. The dominant click frequency and duration of *D. martensii* and *D. deokhatoides* was significantly different ($p < 0.05$) (Table 4.1). The recorded frequency of the low-frequency component did not display any significant difference (Mann-Whitney U-test: Z' = 1.94; $p > 0.05$) between *D. deokhatoides* and *D. martensii*, except for the duration (Z' = -2.93; $p < 0.05$) (Table 4.1).

4.1.2 Head movements and morphology

4.1.2.1 Pipefishes with recorded acoustic signals

All clicks ($n = 94$) produced were observed to be associated with head movement and hyoid depression with the opening of the mouth during feeding strikes.

a) *Doryichthys martensii*

Microtomograph images of the head of *D. martensii* features a prominent 1st postcranial plate (POC1) positioned between the supraoccipital bone (SOC) and 2nd postcranial plate (POC2) (Figure 4.4A, B). The POC1 (equivalent to the coronet in the seahorse) vaguely resembles a blunt arrow head with an anterior end that is slightly grooved and a caudal end that is bifid. The plate rests on respectively the caudal and rostral extensions of the SOC and POC2. Both extensions of the SOC and POC2 are slightly depressed and V-shaped or tapered to the tip. All three bones have a dorsal ridge or carina. Only the dorsal carina of the POC1 runs from anterior to posterior end, while the dorsal carina of both SOC and POC2 runs short of reaching their posterior or anterior

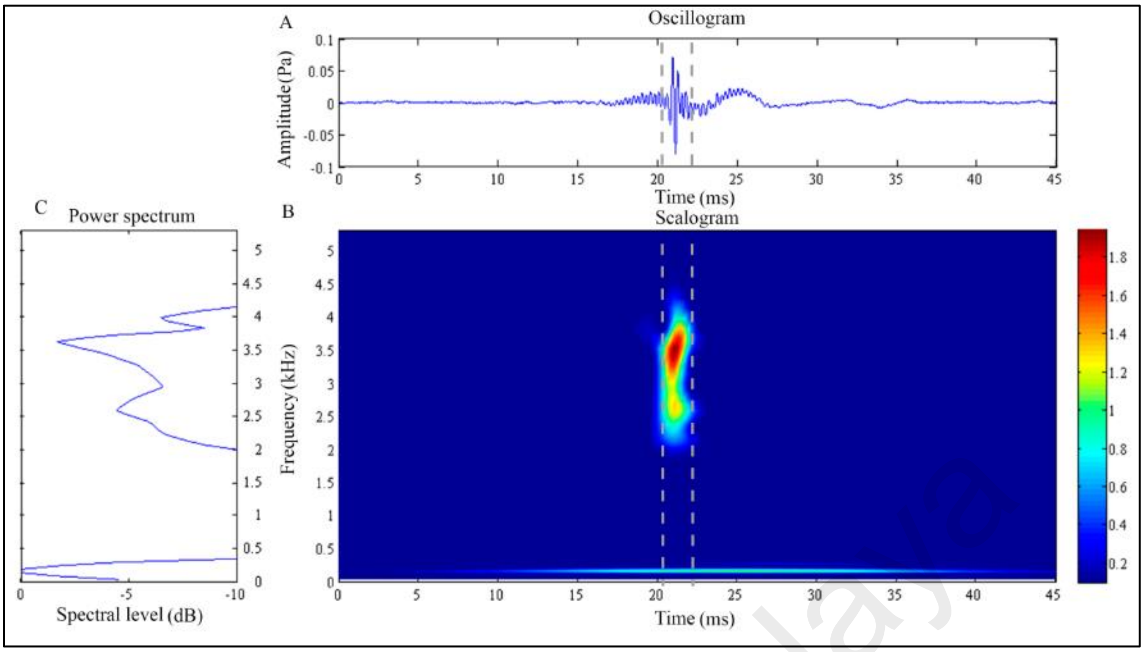

Figure 4.2: (A) Oscillogram, (B) scalogram and (C) energy spectral density of the feeding clicks produced by *Doryichthys deokhatoides*. Vertical lines in (A) and (B) indicate the measured signal duration.

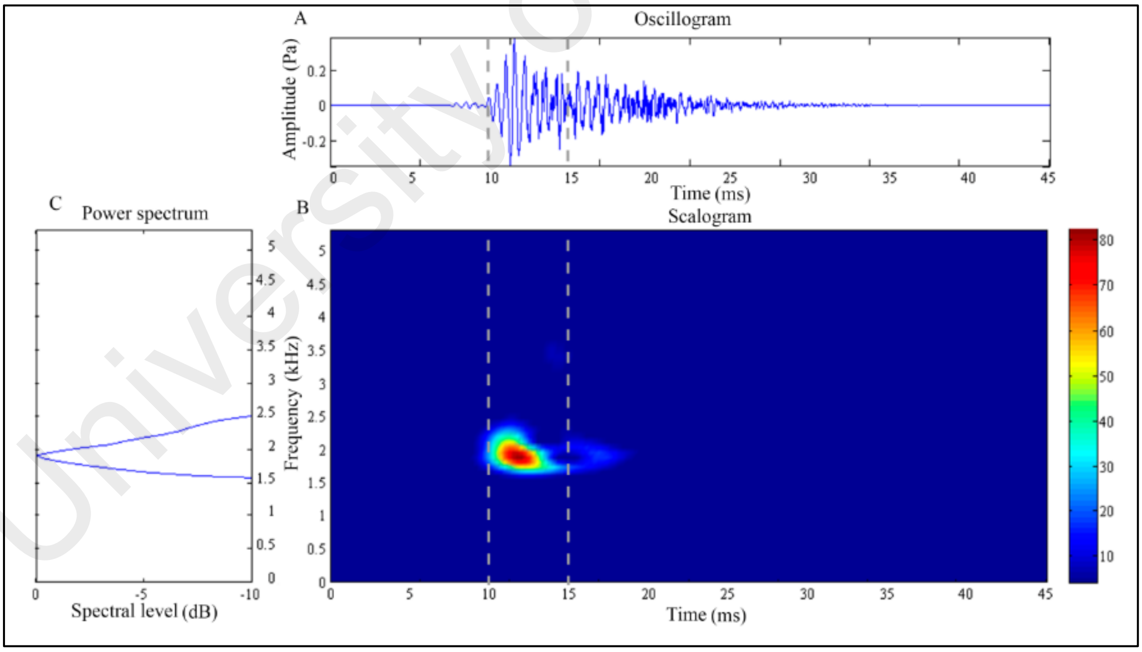

Figure 4.3: (A) Oscillogram, (B) scalogram and (C) energy spectral density of the feeding clicks produced by *Syngnathoides biaculeatus*. Vertical lines in (A) and (B) indicate the measured signal duration.

edge, each ending in a raised, short or blunt spine (Figure 4.4A). The lateral section of *D. martenssi* further revealed that POC1 is not fused to, or articulated with the adjacent bones (Figure 4.4B). The POC1 shows an arched structure resembling a fused double-chevron in transverse section (Figure 4.4C). The long twin sesamoid bones in epaxial tendons (SEM) which run anteriorly from the neck to ligamentously join the SOC are clearly visible beneath the POC1. The arched lateral arms of the POC1 are close to both the post-temporal bone (POSTT) of the cranium and the cleithrum (CL) of the pectoral girdle. The linear system of guided interlocking cranial bone plates which are neither fused nor articulated facilitates bone movements. As the head of the pipefish flexes backwards when the SEM pulls on the SOC, the latter slides beneath the POC1 pushing it towards POC2. The SOC's carina and raised posterior edge provides the "push" against the POC1 while POC2's carina provides the "brake".

b) *Doryichthys deokhatoides*

Similar to the cranial structure of *D. martensii*, the POC1 of *D. deokhatoides* is visibly present between the SOC and POC2 (Figure 4.5A). However, the bone morphology of all three bones differs from that of *D. martensii*. In *D. deokhatoides*, the SOC's dorsal carina ends caudally as a raised blunt spine, while its inferior caudal extension is expanded like a fan with a long medial spine. Both POC1 and POC2 are narrow bone plates located between the twin SEM which run laterally on both sides. The POC1 has more pronounced caudal bifids than in *D. martensii* (Figure 4.5A). The fan-like caudal extension of the SOC of *D. deokhatoides* is tucked underneath the POC1 (Figure 4.5B). Similar to *D. martensii,* backward movements of the head would result in the SOC sliding under the POC1, and the latter (bifids) sliding over the POC2.

c) *Syngnathoides biaculeatus*

Clearing and staining of the head of *S. biaculeatus* revealed the presence of a V-shaped plate at the posterior end of the SOC and on the anterior end of the POC2, just like in *D. martensii*. However, the POC1 plate is distinctly absent leaving a space of about 2 mm between the SOC and POC2 in a fish of 193 cm length (Figure 4.6A). Also clearly different from the two *Doryichthys* species is the absence of a dorsal carina and terminal spine on both the SOC and POC2 of *S. biaculeatus*. The twin SEMs are largely exposed between the SOC and POC2. During head flexion, the SOC is pulled backward by the SEM towards POC2. The large, caudal extension (beak-like) of the SOC thus slides over the rostrum of the POC2 which provides the rough stridulating surface (Figure 4.6B).

4.1.2.2 Pipefishes without recorded acoustic signals

Five other species of pipefishes were examined using histostaining technique to further elucidate the morphology of their sound producing structure. Behavioural observations and sound recording were not performed on these species due to unavailability of live specimens.

a) *Doryichthys boaja*

Histostaining images of *Doryichthys boaja* show the cranial structure to be similar to *D. deokhatoides* by the presence of the POC1 plate between the SOC and POC2 (Figure 4.7A). Both the POC1 and POC2 plates are located between the twin SEM which runs laterally on both sides. The twin SEMs of the *D. boaja* are notably thicker in contrast to the other pipefishes described previously. In *D. boaja*, the caudal end of the SOC ends with an extension that is tucked underneath the POC1 plate (Figure 4.7B). The dorsal end of the POC2 is also observed to be tucked under the POC1. Head flexion would result in

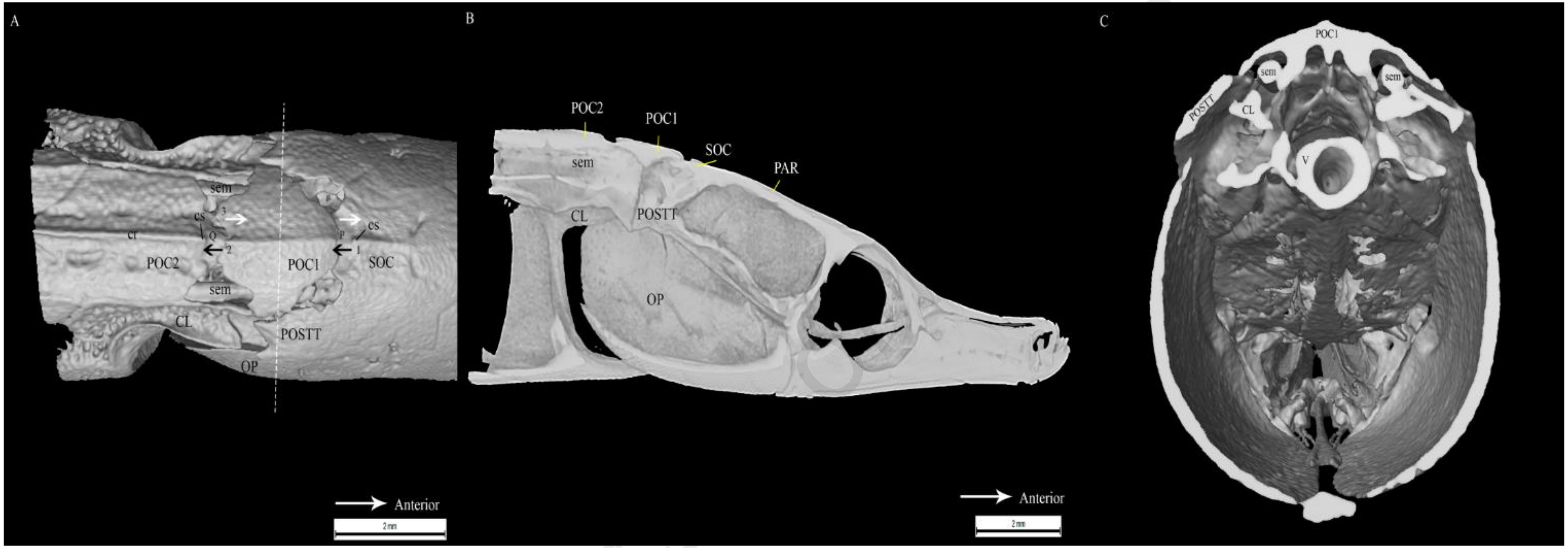

Figure 4.4: Reconstructed tomography images of *Doryichthys martensii* cranium, (A) dorsal aspect, (B) sagittal aspect, and (C) transverse aspect (at POC1, vertical broken line in A), showing morphology of bones (CL – cleithrum; OP – operculum; PAR – parietal; SOC – supraoccipital; POC1 – 1st postcranial; POC2 – 2nd postcranial; POSTT – post-temporal; V – vertebrae column) and ancillary structures (cr – carina ridge or crest; cs– cranial spine; sem – sesamoid bone in epaxial tendon). The three-bone sound producing mechanism initiated during head flexion is hypothesized here; horizontal arrows indicate (1) sliding movement of SOC beneath POC1, first bone strike at P, followed by (2) sliding movement of POC1 above POC2, second bone strike at Q, and (3) return of both POC2 and SOC to their original position after head flexion.

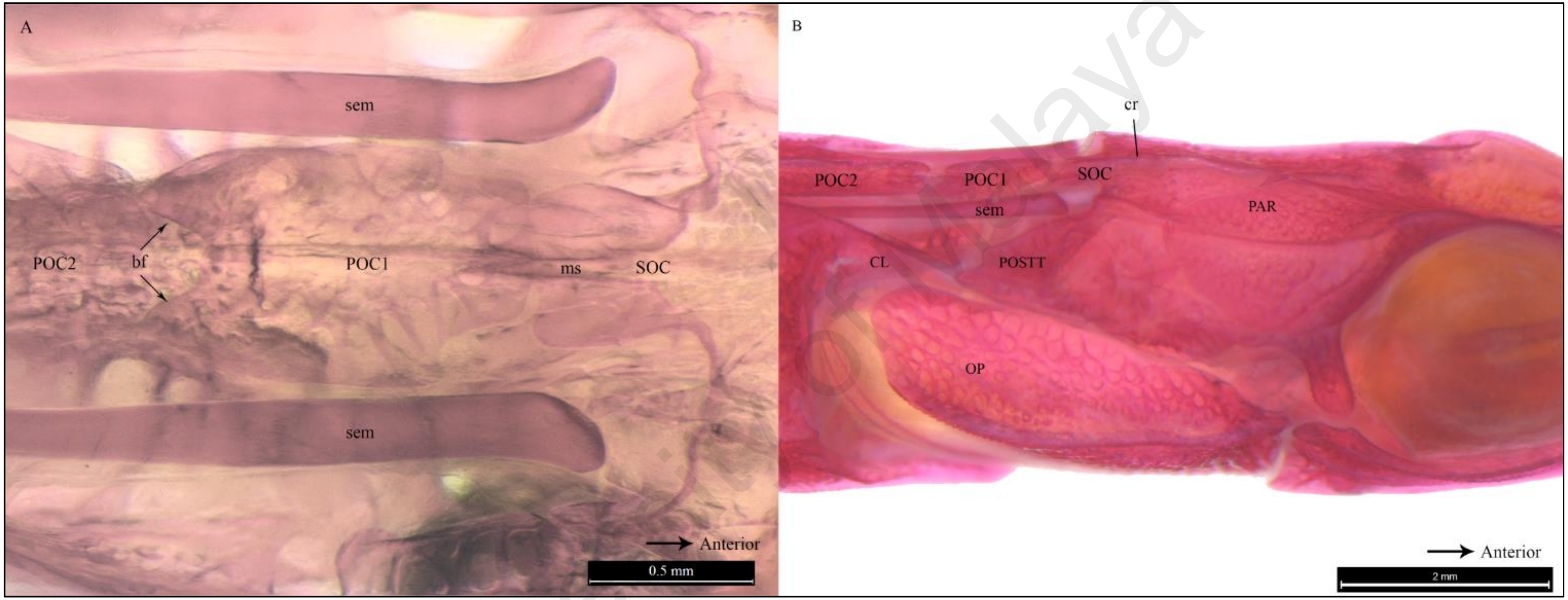

Figure 4.5: Alizarin Red stained images of *Doryichthys deokhatoides* cranium, (A) dorsal aspect and (B) lateral aspect, showing morphology of bones (CL – cleithrum; OP – operculum; PAR – parietal; SOC – supraoccipital; POC1 – 1st postcranial; POC2 – 2nd postcranial; POSTT – post-temporal;) and ancillary structures (cr – carina ridge or crest; cs– cranial spine; sem – sesamoid bone in epaxial tendon; bf – bifid arms of POC1; ms – posterior medial spine of SOC).

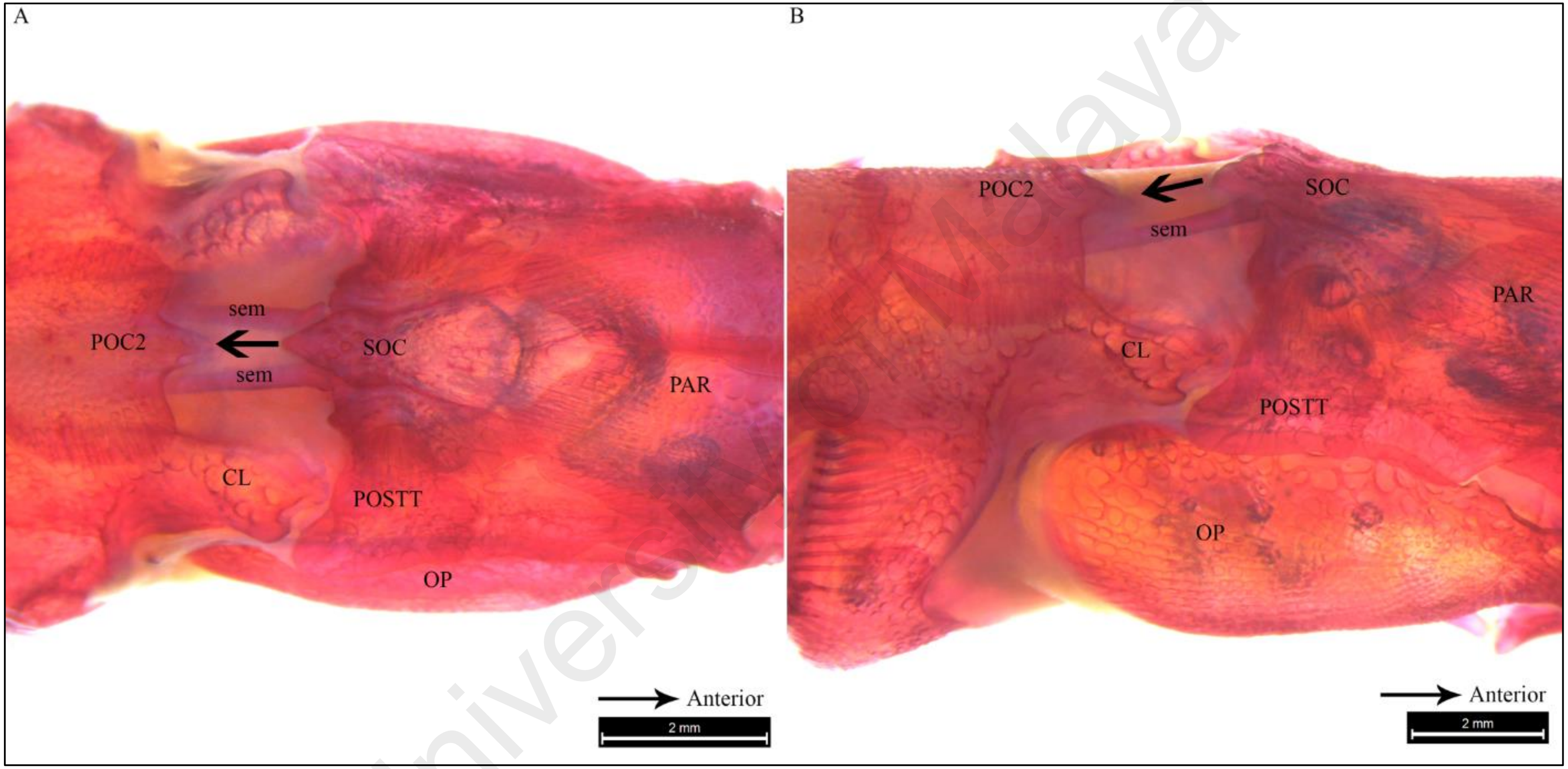

Figure 4.6: Alizarin Red stained images of *Syngnathoides biaculeatus* cranium (A) dorsal aspect and (B) lateral aspect, showing morphology of bones (CL – cleithrum; OP – operculum; PAR – parietal; POC2 – 2nd postcranial; POSTT – post-temporal; SOC – supraoccipital) and the sesamoid bone in epaxial tendon (sem). Note the absence of POC1 as observed in other pipefishes. Arrow indicates the backward movement of SOC which stridulates POC2.

the SOC sliding under the POC1, pushing it over POC2. The visible carina ridge along the centre of the three bones provides the "push" and "brake" similar to *D. deokhatoides*.

b) *Corythoichthys haematopterus*

Similar to the cranial structure of *D. martensii*, the Messmate pipefish, *Corythoichthys haematopterus*, features a prominent POC1 plate positioned between the SOC and POC2 plate (Figure 4.8A). The plate rests on respectively the caudal and rostral extensions of the SOC and POC2. All three plates feature a dorsal ridge or carina that runs from the anterior to posterior end, unlike that of *D. martensii* where only the carinal ridge of the POC1 runs from both ends (Figure 4.8B). Raised blunt knobs and identations were visible on the carinal ridge at the posterior end of the SOC and anterior POC2. Akin to *D. martensii*, the long and slender twin SEMs which run anteriorly from the neck to ligamentously join the SOC are clearly visible beneath the POC1. When the SOC is pulled backwards by the SEMs during head flexion, the SOC slides underneath the POC1 and thrusts it against the POC2. The posterior carina ridge of the SOC pushes against the POC1 while the carina ridge of the POC2 acts as "break" to the backward movements of the POC1C plate (Figure 4.8B).

c) *Trachyrhamphus serratus*

The cranial bones involved in sound production of *Trachyrhamphus serratus* is similar to *D. deokhatoides* and *D. boaja* in that the narrow POC1 and POC2 plates are located in between twin SEM bones (Figure 4.9A, B). The posterior end of the SOC was tapered and joined by the twin SEM at the epaxial ligaments, while the POC1 resembles a diamond with pointed ends (Figure 4.9 B). The dorsal carinal ridge runs from the anterior to the posterior ends of the POC1 and POC2 plates, but they are not pronounced as in the SOC. Similar to *D. deokhatoides* and *D. boaja*, backward movement of the head result in the SOC sliding under the POC1, and the latter sliding over the POC2.

d) *Dunckerocampus dactyliophorus*

The banded pipefish, *Dunckerocampus dactyliophorus* comes under the subfamily Doryhamphinae (flag-tail pipefishes), whereas the described cranial structure of the previous six species falls under the subfamily Syngnathinae (true pipefishes). Despite coming under a different subfamily, the cranial structure of *Dunckerocampus dactyliophorus* resembles that of *D. deokhatoides*, *D. boaja* and *T. serratus* with the POC1 and POC2 plate located between the two SEM bones (Figure 4.10 A, B). Despite adopting similar cranial morphological arrangements, the bone morphology of the aforementioned bone plates differs from that of *D. deokhatoides*, *D. boaja* and *T. serratus*. The SOC features a thick caudal extension that is ligamentously connected to the twin SEMs whereas the POC1 plate possesses similar caudal bifids as the *D. deokhatoides* but they are less pronounced. The anterior end of the POC2 is similar to the anterior of the POC1 plate, featuring a rounded edge. The pair of SEMs in the *Dunckerocampus dactyliophorus* are notably longer than the other described pipefishes. Additionally, the twin SEMs were also observed to be thicker, like *D. boaja*, when compared to the other examined pipefish species. Unlike the described carinal ridge of the other pipefishes, the carinal ridge of *Dunckerocampus dactyliophorus* is either absent or undistinguishable. However, all three bone pieces features visible “studs” on its surface (Figure 4.10B). The SOC will slide under the POC1 and the bifid posterior end of the POC1 sliding over the POC2 plate during head flexion (Figure 4.10 A).

e) *Acentronura tentaculata*

The pygmy pipehorse, *Acentronura tentaculata* comes under the subfamily Solegnathinae (pipehorse). Fascinatingly, the histostaining images of the cranial structure of *A. tentaculata* (Figure 4.11A, B) reveal a different cranial arrangement compared to the previously described syngnathid species. Despite having the POC1 plate in between the twin short slender SEMs as observed in other species (*D. deokhatoides, D. boaja, Dunckerocampus dactyliophorus* and *T. serratus*) (Figure 4.11B), the posterior SOC and anterior POC2 plate forms a unique beak as observed in *S. biaculeatus*. Both the posterior and anterior ends of the SOC and POC2 bones are tapered, similarly to that the SOC and POC2 bones observed in *S. biaculeatus*. Another noteworthy observation is the position of the POC1 plate which appears to be a reduced narrow form with its posterior end located under the POC2 bone. Uniquely, a ventral protrusion is observed caudally beyond the tapered extension of the SOC. Based on the histochemical images, this protrusion appears to be part of the SOC with a visible carine ridge on the protrusion in contrast to the dorsal SOC which is without it. The carina ridge is also present on the POC2 plate, while carina spine is not visibly present in this species (Figure 4.11 B). When the head of the pipehorse is pulled back by the short twin SEMs during flexion, the tapered posterior end of the SOC meets the anterior end of the POC2, crossing over the POC1 plate. Subsequently, the POC2 plate provides the stridulating surface since its dorsal surface is rough with raised carinal ridge. The ventral exposed part of the SOC and the POC1 appears to be vestigial structures and does not meet during head flexion (Figure 4.11 A).

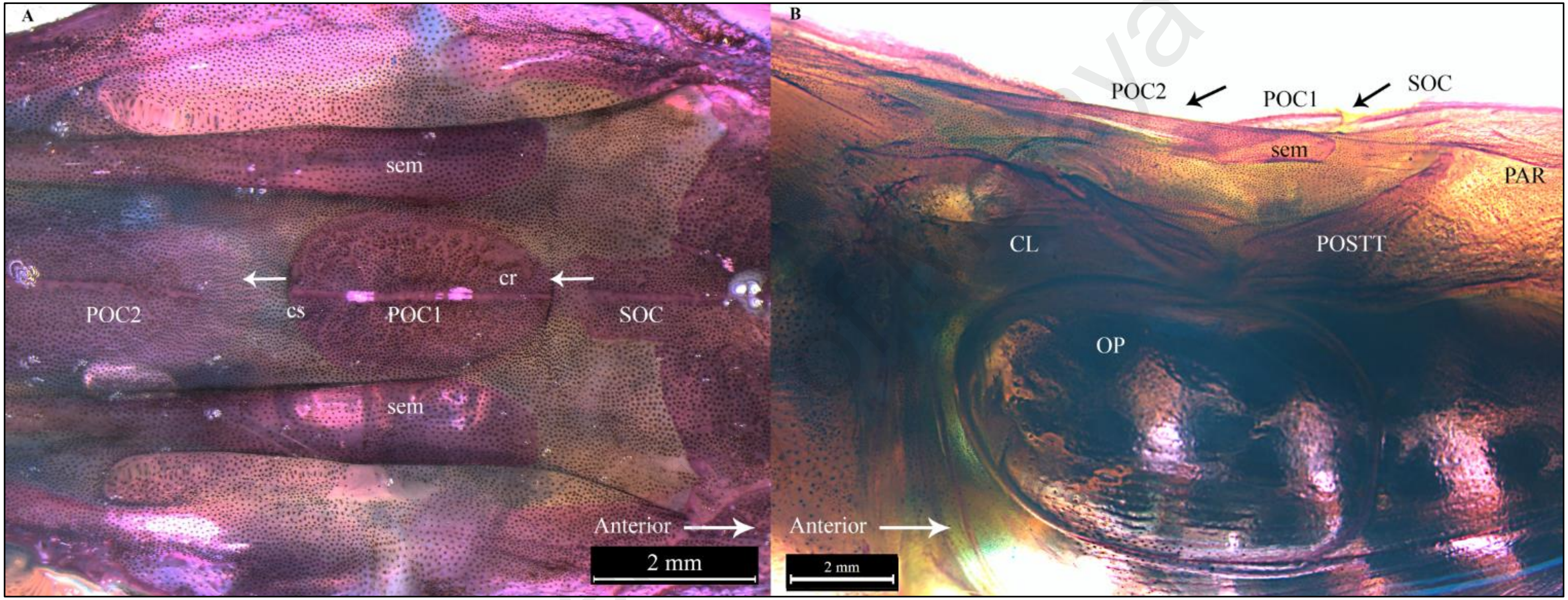

Figure 4.7: Alizarin Red stained images of *Doryichthys boaja* cranium, (A) dorsal aspect and (B) lateral aspect, showing morphology of bones (CL – cleithrum; OP – operculum; PAR – parietal; SOC – supraoccipital; POC1 – 1st postcranial; POC2 – 2nd postcranial; POSTT – post-temporal;) and ancillary structures (cr – carina ridge or crest; cs– cranial spine; sem – sesamoid bone in epaxial tendon). Arrow indicates the backward movement of SOC striking the POC1, pushing it backwards to strike the POC2.

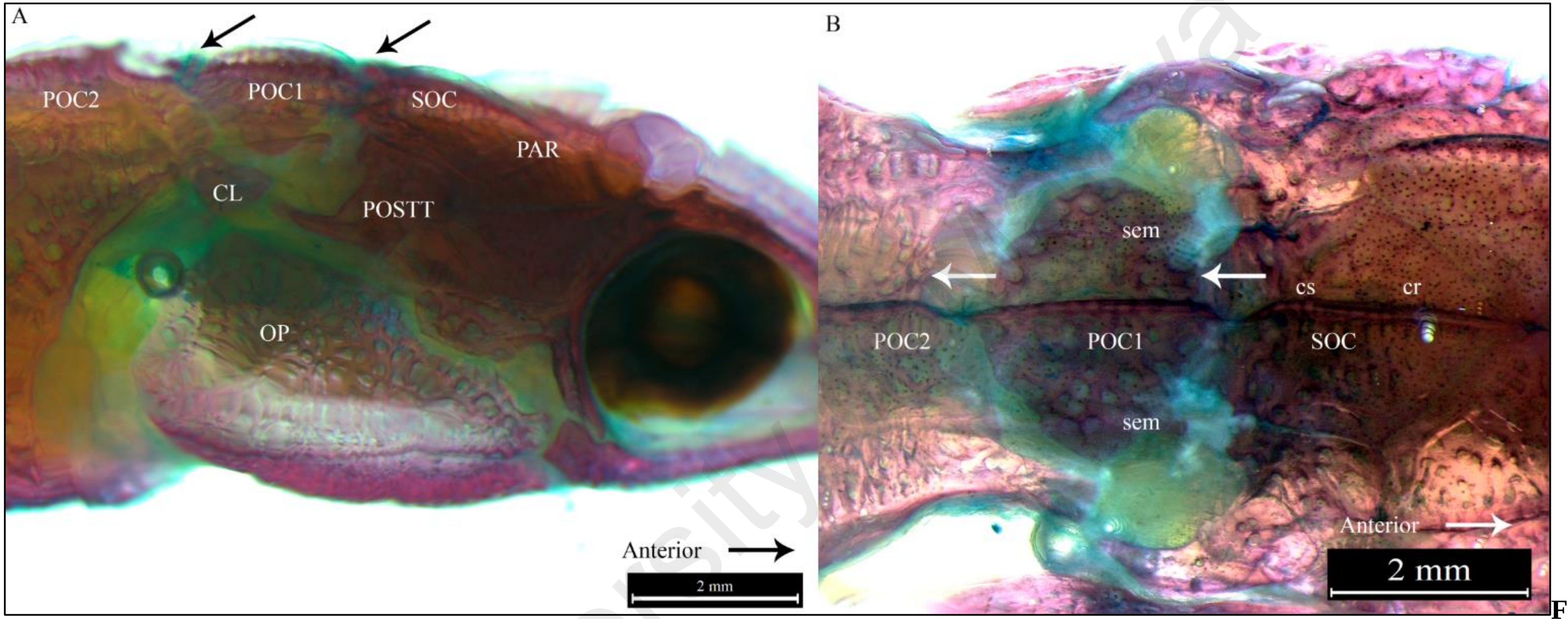

Figure 4.8: Alizarin Red stained images of *Corythoichthys haematopterus* cranium, (A) lateral aspect and (B) dorsal aspect, showing morphology of bones (CL – cleithrum; OP – operculum; PAR – parietal; SOC – supraoccipital; POC1 – 1st postcranial; POC2 – 2nd postcranial; POSTT post-temporal;) and ancillary structures (cr – carina ridge or crest; cs– cranial spine; sem – sesamoid bone in epaxial tendon). Arrow indicates the backward movement of SOC striking the POC1, pushing it backwards to strike the POC2.

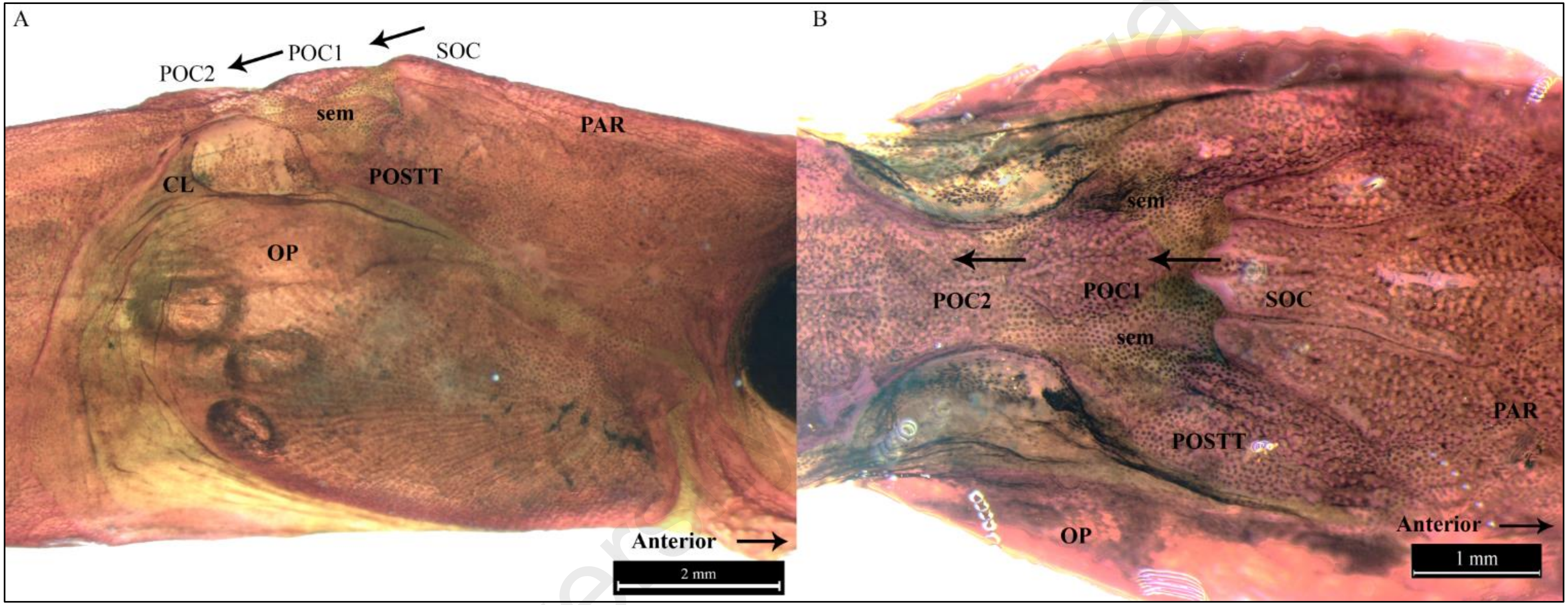

Figure 4.9: Alizarin Red stained images of *Trachyrhamphus serratus* cranium, (A) lateral aspect and (B) dorsal aspect, showing morphology of bones (CL – cleithrum; OP – operculum; PAR – parietal; SOC – supraoccipital; POC1 – 1st postcranial; POC2 – 2nd postcranial; POSTT – post-temporal;) and the sesamoid bone in epaxial tendon (sem). Arrow indicates the backward movement of SOC striking the POC1, pushing it backwards to strike the POC2.

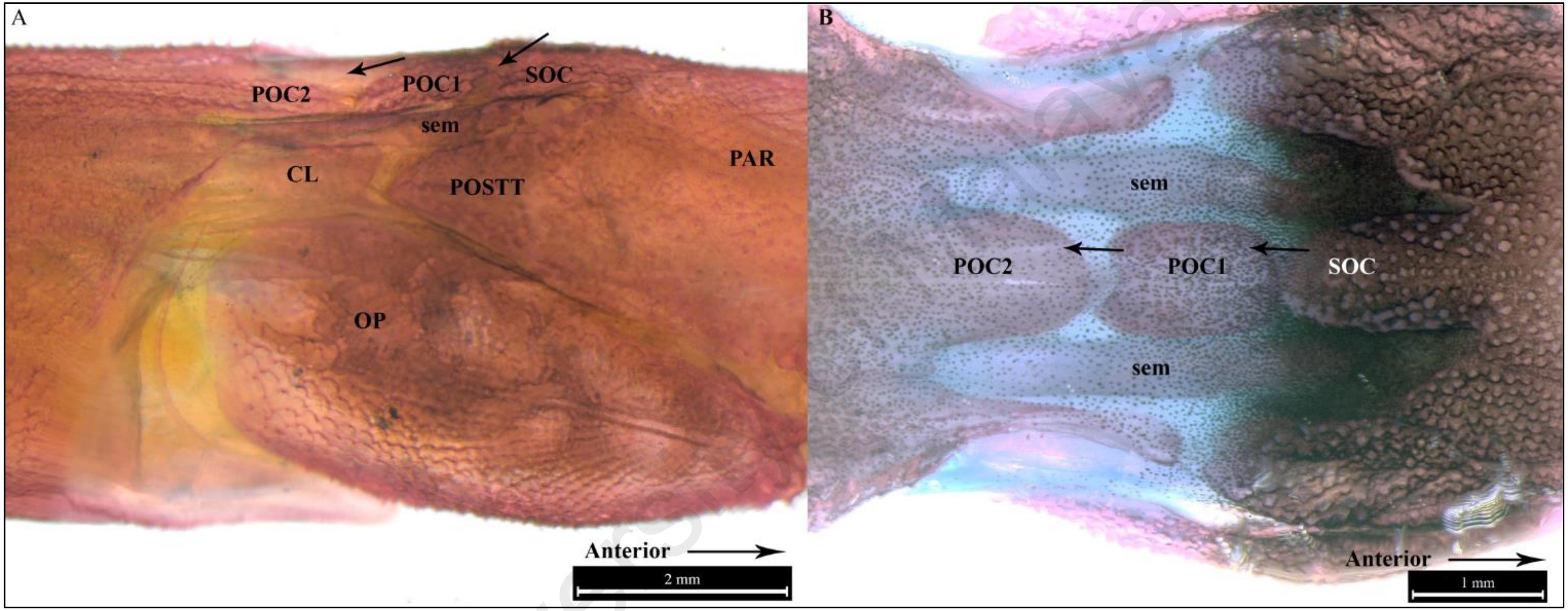

Figure 4.10: Alizarin Red stained images of *Dunckerocampus dactyliophorus* cranium, (A) lateral aspect and (B) dorsal aspect, showing morphology of bones (CL – cleithrum; OP – operculum; PAR – parietal; SOC – supraoccipital; POC1 – 1st postcranial; POC2 – 2nd postcranial; POSTT – post-temporal;) and the sesamoid bone in epaxial tendon (sem). Arrow indicates the backward movement of SOC striking the POC1, pushing it backwards to strike the POC2.

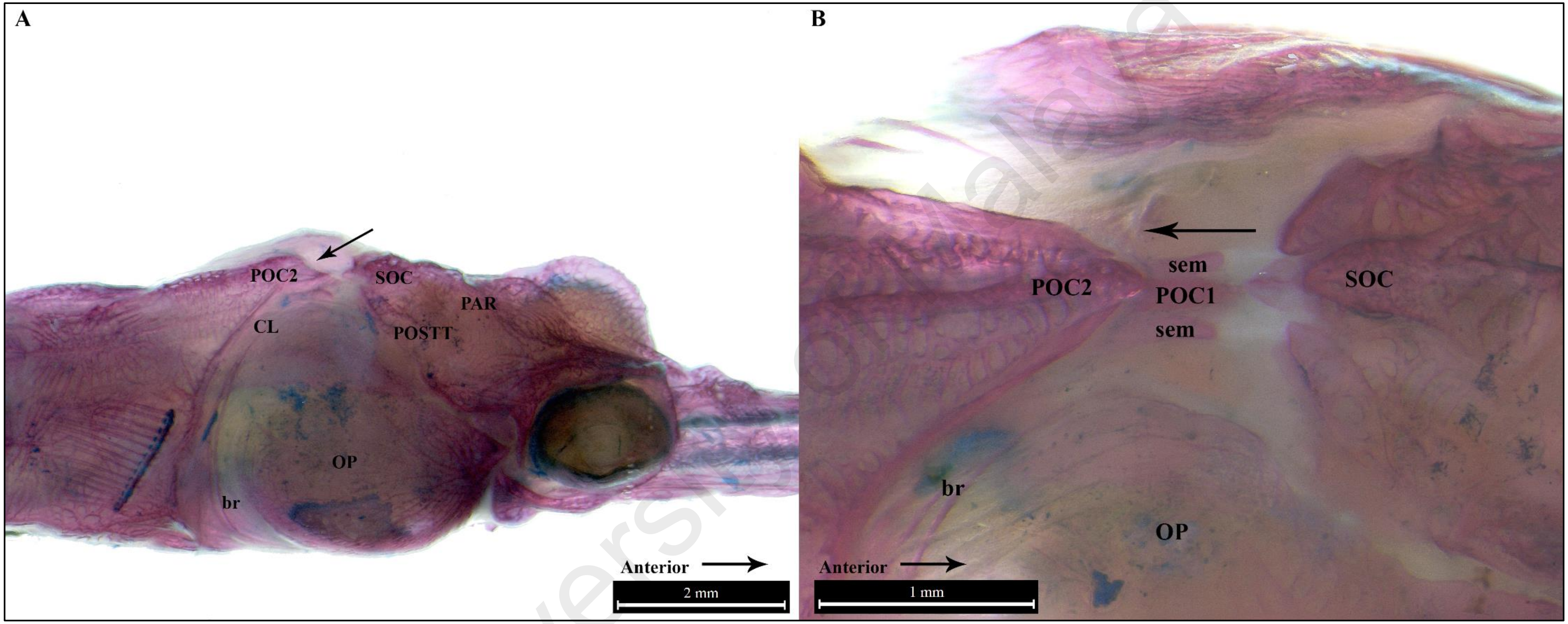

Figure 4.11: Alizarin Red stained images of *Acentronura tentaculata* cranium, (A) lateral aspect and (B) dorsal aspect, showing morphology of bones (CL – cleithrum; OP – operculum; PAR – parietal; SOC – supraoccipital; POC1 – 1st postcranial; POC2 – 2nd postcranial; POSTT – post-temporal;) and ancillary structures (br – branchiostegal ray; cr – carina ridge or crest; sem – sesamoid bone in epaxial tendon). Arrow indicates the backward movement of SOC which stridulates POC2.

4.2 Discussion

The click signal waveform of *Doryichthys* pipefishes is consistent with that of other species and their close relative, the seahorse, in depicting an initial burst of energy followed by a rapid decay of signal energy (Colson *et al.*, 1998; Ripley and Foran, 2007; Chakraborty *et al.*, 2014; Haris *et al.*, 2014; Oliveira *et al.*, 2014; Lim *et al.*, 2015). However, the signal waveform of *S. biaculeatus* depicts a multimodal sinusoidal waveform unlike the sharp energy spikes recorded in the *Doryichthyes* pipefishes. The dominant frequencies and duration of clicks of all three pipefish species generally displayed no intraspecific differences and are consistent with those reported for *Syngnathus floridae* and *Syngnathus fuscus* (Ripley and Foran, 2007). It has however been documented that interspecific differences were observed in recorded clicks of other pipefishes such as *Syngnathus floridae* and *Syngnathus fuscus* (Ripley and Foran, 2007). In the present study, all three species are different in their dominant click frequency. However, the click duration is not different between *S. biaculeatus* and *D. martensii* but both are different from *D.* martensii. Nevertheless, the produced scalograms of all three species displayed species-specific spectral forms which were quantitatively consistent within species. Thus, wavelet analysis together with sound characteristics values could be a useful tool for species differentiation based on sound.

The possibility of interspecific differentiation in sound characteristics among seahorse species has also been suggested previously (see Chapter 4). Sound diversity within the same family has also been reported in other fish families which may allow species recognition (Amorim, 2006; Kaatz *et al.*, 2010; Fine and Parmentier, 2015). Nevertheless, there are other sources of sound variability such as fish size, ontogeny and sex (Amorim, 2006) which are not covered in the present study. For instance, a negative correlation between frequency peaks and fish size in the seahorse, *Hippocampus zosterae* has been reported (Colson *et al*., 1998), while there are sexual differences in the sound characteristics of *Hippocampus reidi* (Oliveira *et al*., 2014).

Microtomography reveals similarity in the cranial bones associated with sound production in the *Doryichthyis* genus, *Duncekerocampus dactyliophorus, C. haematopterus* and *T. serratus* in that a POC1 bone plate is present between the SOC and POC2 plates, depicting a linearly arranged three-bone mechanism (SOC-POC1-POC2). However, this mechanism is modified in *S. biculeatus* where POC1 is clearly absent, or reduced as in *A. tentaculata*. Despite the similarity in the *Doryichthyis* genus, *Duncekerocampus dactyliophorus, C. haematopterus* and *T. serratus*, the size and morphology of the associated bones differs between the species which may produce different sound signals or their patterns. The cranial morphological arrangement of the eight species above can generally be categorised into four separate groups, those with (A) POC1 over SEM, (B) POC1 between SEM, (C) absent POC1 and (D) reduced POC1 (Figure 4.12).

The sesamoid bone in the epaxial tendons were observed in all eight species of pipefish consistent with previous reports on other species which are the *Dunckerocampus pessuliferus, Doryhamphus janssi* and *Corythoichthys insularis* (Leysen *et al*., 2011). In both the pipefish and seahorse, the twin sesamoid bones, epaxial tendons and muscles provide the traction power that pulls the SOC backwards during head flexion whereas the sesamoid bones are formed as protection for tendons that passes over a joint (Sarin *et al*., 1999; Hall, 2005; Van Wassenbergh *et al.,* 2008; Leysen *et al.,* 2011). The backward slide of the SOC initiates stridulations and bone strikes between the SOC and postcranial plates behind it. The three-bone mechanism provides two possible successive strikes between the kinetic cranial bones for the first two groups (Figure 4.12 A, B). In the case of the third and fourth group (*S. biculeatus* and *A. tentaculata*), in the absence or reduced POC1, a stridulatory movement between the SOC and POC2 is produced and there appears to be no bone strike from the sound waveform produced (Figure 4.12 C, D). The much amplified sinusoidal waveform of *S. biaculeatus* feeding click could be attributed to the longer distance between the SOC and the POC2 when the two bones stridulate. Stridulation rather than knocking between cranial bones is more likely given the bone morphology including surface roughness, and the greater distance between bones to generate greater energy.

More evidence of stridulation in the third group is gleaned from the generated waveform of *S. biaculeatus* (Figure 4.3A) which closely resembles that of the blue catfish when the pectoral spine rubs against the pelvic girdle (Mohajer *et al.*, 2015). However, there is a difference in the waveform; it is multimodal in the Alligator pipefish but unimodal in the blue catfish. In the Alligator pipefish, the pulse appears to result from a more forceful backward slide of the SOC over the POC2. The Alligator pipefish's click waveform in fact agrees with the general description of the stridulatory mechanism which produces an assemblage of irregular transient pulses of a wide range of frequencies (Hawkins, 1993). Additionally, the low frequency component was not present in the Alligator pipefish. The bones of the SOC and POC2 are further apart with the absence of the POC1 unlike that of other pipefishes. It is not clear why *S. biaculeatus* does not display this low-frequency component although one reason could be connected to the absence of the POC1. It is speculated that the sliding between SOC and POC1 bones basically produces a short band signal which may not stimulate resonance of the girdle.

Waveforms produced by stridulation and forceful strike (knock) between bones and appearing in that order are clearly evident in *D. deokhatoides* (see Figure 4.2) consistent with the three-bone sound producing mechanism. The low frequency component in *D. deokhathoides* (see figure 4.2) is probably a by-product of the sliding movement of the medial spine of the SOC prior to contact of the dorsal carinal spine (SOC) with the POC1 (see Figure 4.5). Interestingly, the seahorse which makes a single and forceful bone strike also produces an energy burst of high-frequency sound but this involves the highly modified and elevated SOC and POC1 (or coronet) plate (Colson *et al.,* 1998; Leysen *et al.,* 2011; Chapter 4). Thus, the differences in cranial bone morphology likely explain the different sound signals or their patterns as observed in the pipefishes. Despite the pygmy pipehorse, *A. tentaculata*, revealing a structurally different arrangement (Figure 4.11B), it can be grouped along with *S. biaculeatus* (alligator pipefish) as with "absent POC1". Upon closer inspection of the cranial structure of the pipehorse, the "beaks" of the posterior SOC and anterior POC2 along with the narrow twin SEM closely resembles that of the Alligator pipefish (Figure 4.6) with the exception of the narrow POC1. The narrow POC1 plate is positioned underneath the POC2 plate and appears to be a vestigial bone piece when compared to the POC1 plate of other pipefishes (Figure 4.11A, B). In addition, forced movement of the sample under the microscope revealed that the POC1 does not make contact with the SOC during head flexion. The POC1 bone has been reduced and apparently no longer serves its function in *A. tentaculata*. It is not known what other function (if any) it may now serve in relation to sound production.

The wavelet analysis also reveals a low-frequency sinusoidal component present in most of the recorded feeding clicks of *D. martensii*, which is only consistent in one individual of *D. deokhatoides*, and none in *S. biaculeatus*. This low-frequency component has never been reported before. Previous works did not detect this component because the methods employed only revealed the click waveforms (oscillogram) which

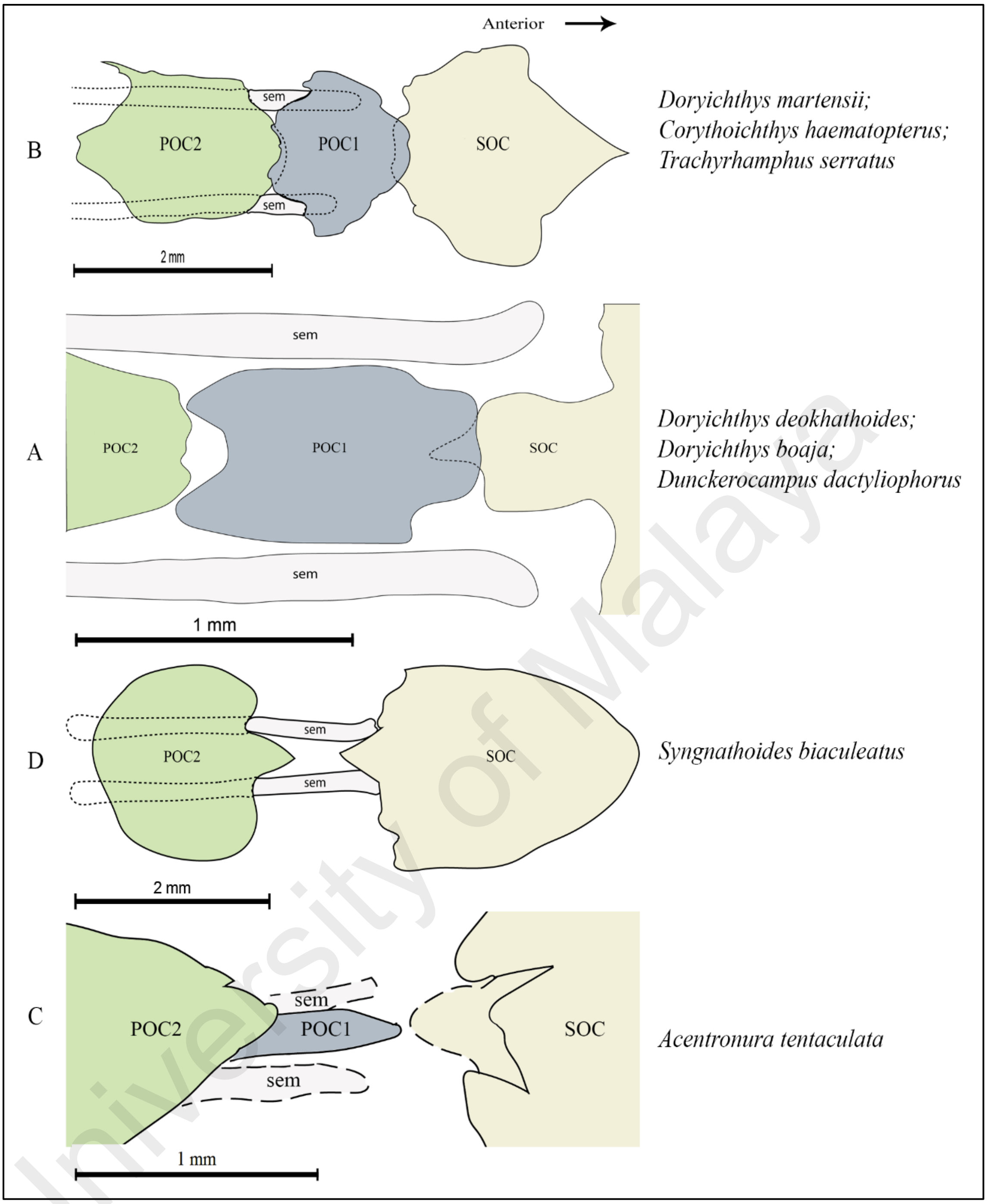

Figure 4.12: The morphological arrangement of different groups of sound producing structures, A) 1st postcranial plate over sesamoid bones in epaxial tendon, B) 1st postcranial plate between sesamoid bones in epaxial tendon, C) 1st postcranial plate absent and D) 1st postcranial plate reduced (SOC, supraoccipital; POC1, 1st postcranial; POC2, 2nd postoccipital; SEM, sesamoid bones in epaxial tendon).

temporal pattern of the low frequency component was found to be different than the dominant higher frequency component. A similar low-frequency component which was also documented in the feeding click of seahorses was documented as a compounded sound produced by two mechanisms, one by the cranial bones and the other by stochastic resonance of the pectoral girdle (see Chapter 3). Interestingly, the temporal pattern of the low-frequency component matches that as reported in seahorses, which has a frequency range of 150 – 200 Hz. Thus, it is probable that the low-frequency sound component in both *Dorichythes* pipefishes is a secondarily-derived sound produced by the pectoral girdle and stimulated by the cranial bones, given the close proximity of POC1 and the cleithrum. In *D. deokhatoides*, these bones are further apart. It is not clear however, as to why *S. biaculeatus* does not display this low-frequency signal although one morphological reason could be connected to the absence of the POC1 plate. However, the more likely reason is that the sliding between the SOC and POC1 bones basically produces a narrow band signal which may not stimulate resonance of the girdle.

In summary, the feeding clicks of pipefishes display a localised energy distribution with interspecific differences and unique spectral signatures. The differences in cranial bone morphology (i.e. those associated with sound production) appear to be an important factor in the production of species-specific signatures. Such varied morphologies and acoustic signatures may benefit species identification. Thus, the results support the hypothesis of sound producing mechanisms differing among species and the hypothesis of species-specific signatures. However, the results from this study are inadequate to demonstrate clear individualistic or intraspecific signatures. The varied acoustic signatures may have significance in interspecific communication in pipefishes which however requires further work.

CHAPTER 5: DIVERSITY, HABITAT AND CONSERVATION THREATS OF SYNGNATHID FISHES IN MALAYSIA

5.1 Results

5.1.1 Species Richness

High species richness with a total of 56 species (Table 5.1) encompassing all four subfamilies of Syngnathidae, was recorded from Malaysian waters. From the total are 35 species of pipefishes (Syngnathinae), five species of flag-tail pipefishes (Doryrhamphinae), three species of pipehorses (Solegnathinae) and 13 species of seahorses (Hippocampinae); encompassing 19 different genera with no new syngnathid species were discovered in this study. The region with the most diverse syngnathid fauna is Sabah with a total of 42 species, followed by Peninsular Malaysia with 33 species and Sarawak with only nine species. Highest species diversity is located within the Semporna Islands of Sabah with no fewer than 26 species of syngnathids.

Table 5.1: Compilation of Malaysian syngnathid species from present and previous studies. IUCN status (LC = Least concern; DD = Data deficient; NT = Near threatened; VU = Vulnerable; EN = Endangered; CE = Critically endangered and NE = Not evaluated). (*) = Collected in present study, AS = Anecdotal records.

Species	Habitat	Locality	Present Study	Others
Subfamily Doryhamphinae				
Dunckerocampus pessuliferus	Estuary	Semporna Islands	*	DAWSON 1985
(Fowler, 1938)$^{\text{NE}}$	Coastal / Shallow waters			
	Deep waters			
Dunckerocampus dactyliophorus	Coastal / Shallow waters	Perhentian / Redang	*	WOOD and WOOD 1987
(Bleeker, 1853)$^{\text{DD}}$		Semporna Islands	*	ALLEN 1992; USNM
		Tunku Abdul Rahman Park		USNM; FISHWISE 2007
		Sugud Islands	*	RG
		Malaysia		KPM
Doryrhamphus excisus excisus	Coastal / Shallow waters	Perhentian / Redang	*	
Kaup, 1856$^{\text{NE}}$		Semporna Islands	*	ALLEN 1992
Doryrhamphus janssi	Coastal / Shallow waters	Perhentian / Redang	*	
(Herald and Randall, 1972)$^{\text{LC}}$		Payar / Langkawi	*	
		Mersing Islands	*	
		Tiga Islands	*	
Doryrhamphus negrosensis negrosensis	Coastal / Shallow waters	Semporna Islands	*	ALLEN 1992; USNM
Herre, 1934$^{\text{NE}}$		Sabah		GCRL
		Lahad Datu		USNM
		Banggi Islands	*	KPM

Table 5.1 continued

Species	Habitat	Locality	Present Study	Others
Subfamily Hippocampinae				
Hippocampus barbouri	Macroalgae	Sandakan		USNM; CAS
Jordan and Richardson, 1908^{VU}		Sabah		CHOO and LIEW 2004
		Banggi Islands		LOURIE et al. 2005
Hippocampus bargibanti	*Muricella* seafan	Semporna Islands	*	
Whitley, 1970^{DD}		Spratly Islands	*	
Hippocampus comes	Coastal / Shallow waters	Pulai River		CHOO et al. 2009
Cantor, 1850^{VU}	Macroalgae	Penang	*	BMNH; LOURIE et al. 2004
	Seagrass	Payar, Langkawi	*	
		Mersing Islands		MORGAN and LOURIE 2006
		Kudat		
		Lahad Datu		CHOO and LIEW 2004
		Tunku Abdul Rahman Park		CHOO and LIEW 2004 FISHWISE 2007
Hippocampus denise	*Annelia*, *Muricella* and *Echinogorgia* seafan	Spratly Islands	*	
Lourie and Randall, 2003^{DD}		Semporna Islands	*	LOURIE and RANDALL 2003
Hippocampus histrix	Seagrass	Jarak Island	*	
Kaup, 1856^{DD}	Macroalgae	Sulu Sea, Sabah		CHOO and LIEW 2004
		Mukah		CHOO and LIEW 2004
		Sugud Islands		RG
		Semporna Islands	*	KUITER 2009
Hippocampus kelloggi	Coastal / Shallow waters	Tukun Tun Perak		CHOO and LIEW 2003
Jordan and Snyder, 1902^{DD}	Deep waters	Sabah		CHOO and LIEW 2004
		Belawai, Sarawak		CHOO and LIEW 2004

Table 5.1 continued

Species	Habitat	Locality	Present Study	Others
Hippocampus kuda	Estuary	Tanjung Tuan, Malacca	*	
Bleeker, 1852^{VU}	Macroalgae	Pulai River	*	CHOO and LIEW 2003; CAS
	Seagrass	Kuala Skudai		BMNH
	Coastal / Shallow waters	Perepat River		UBC
		Johor River	*	LOURIE et al. 2005
		Kota Kinabalu		CHOO and LIEW 2004
		Lahad Datu		CHOO and LIEW 2004
		Inanan River		UMS
		Sandakan		LOURIE et al. 2005
Hippocampus mohnikei	Seagrass	Penang	*	
Bleeker, 1853^{DD}	Estuary	Matang	*	
Hippocampus satomiae	Soft corals	Semporna Islands	*	LOURIE and KUITER 2008
Lourie and Kuiter, 2008^{DD}				
Hippocampus severnsi	Soft corals	Semporna Islands	*	
Lourie and Kuiter, 2008^{DD}				
Hippocampus spinosissimus	Macroalgae	Peninsular Malaysia	*	CHOO and LIEW 2003
Weber, 1913^{VU}	Coastal / Shallow waters	Sabah and Sarawak	*	CHOO and LIEW 2004
	Deep waters	Santubong		LOURIE et al. 2005
		Banggi Islands		LOURIE et al. 2005
		Labuan		LOURIE et al. 2005
		Spratly Islands		LOURIE et al. 2005
Hippocampus trimaculatus	Estuary	Peninsular Malaysia	*	CHOO and LIEW 2003
Leach, 1814^{VU}	Coastal / Shallow waters	Sabah and Sarawak	*	CHOO and LIEW 2004
		Sembilan Islands / Pangkor		LOURIE and VINCENT 2004
		Penang		FOWLER

Table 5.1 continued

Species	Habitat	Locality	Present Study	Others
		Santubong		LOURIE et al. 2005
Kyonemichthys rumengani	Coastal / Shallow waters	Semporna Islands	*	
Gomon, 2009^{NE}				
Subfamily Solegnathinae				
Acentronura tentaculata	Seagrass	Mersing Islands	*	
Günther, 1870^{NE}				
Solegnathus hardwickii	Deep waters	Perhentian/ Redang	AS	
(Gray, 1830) ^{DD}		Kota Kinabalu	*	
Solegnathus lettiensis	Deep waters	Sabah		MOHSIN and AMBAK 1996
Bleeker,1860^{DD}		Malaysia		FISHWISE 2007
Subfamily Syngnathinae				
Choeroichthys brachysoma	Seagrass	Semporna Islands		KUITER 2009
(Bleeker, 1855)^{NE}	Coastal / Shallow waters			
Corythoichthys amplexus	Coastal / Shallow waters	Perhentian/ Redang	*	
Dawson and Randall, 1975^{LC}		Kapas Island	*	
		Mersing Islands	*	
		Tiga Islands	*	
Corythoichthys flavofasciatus	Coastal / Shallow waters	Mersing Islands	*	
(Rüppell, 1838)^{NE}		Semporna Islands	*	
		Spratly Islands	*	
Corythoichthys haematopterus	Coastal / Shallow waters	Perhentian / Redang	*	HARBORNE et al. 2000
(Bleeker, 1851)^{NE}		Semporna Islands	*	ALLEN 1992
		Spratly Islands		PILCHER et al. 1999

Table 5.1 continued

Species	Habitat	Locality	Present Study	Others
		Malaysia		BMNH; MNHN; KPM
Corythoichthys intestinalis	Coastal / Shallow waters	Semporna Islands	*	DAWSON, 1985
(Ramsay, 1881)^{NE}		Lahad Datu		USNM
Corythoichthys schultzi	Coastal / Shallow waters	Semporna Islands	*	
Herald, 1953^{NE}		Spratly Islands	*	
		Malaysia	*	
Cosmocampus banneri	Coastal / Shallow waters	Semporna Islands		ALLEN 1992
(Herald and Randall, 1972)				
Doryichthys boaja	River	Pulai River	*	
(Bleeker, 1851)^{NE}	Stream	Klang		ZAKARIA –ISMAIL 1989
		Muar		ZAKARIA –ISMAIL 1989
		Peninsular Malaysia		DAWSON 1985
		Malacca		FOWLER 1938
		Kuap River, Sarawak		ATACK 2006
		Sarawak		BMNH, SMK
		Sulaman River		BMNH; USNM
Dorythoichthys deokhatoides	River	Mawai		HERRE and MYERS 1937; BMNH
(Bleeker, 1853)^{NE}	Stream			
		Muar		USNM
		Ayer Puteh		USNM
		Endau Rompin		NG and TAN 1999
		Selangor		BISHOP 1973; MNMH
		Kahang River	*	
		Kota Tinggi		DUNCKER 1904; DAWSON 1985

Table 5.1 continued

Species	Habitat	Locality	Present Study	Others
		Kejin River		ROM; BMNH
Dorythoichthys martensii	River	Mawai		HERRE 1940
(Peters, 1869)^{NE}	Stream	Kota Tinggi		DAWSON 1985
		Tapah		SU
		Simpang Renggam		SU
		Kuala Berang		BMNH
		Tersat		BMNH
		Kahang River	*	
		Perak River		HERRE 1940
		Selangor		MOHSIN and AMBAK 1983
		Pahang		ZAKARIA-ISMAIL 1989
		Rajang Basin		PARENTI and LIM 2005
Festucalex erythraeus	Coastal / Shallow waters	Semporna Islands		DAWSON 1985
(Gilbert, 1905)^{NE}				
Halicampus brocki	Coastal / Shallow waters	Semporna Islands	*	
(Herald, 1953)^{NE}		Malaysia		KPM
Halicampus grayi	Coastal / Shallow waters	Banggi Islands	*	
Kaup, 1856^{NE}				
Halicampus macrorhynchus	Coastal / Shallow waters	Mersing Islands	*	
Bamber, 1915^{NE}		Semporna Islands	*	
Hippichthys cyanospilos	Rivers	Kuala Sepetang	*	
(Bleeker, 1854)^{NE}	Estuary	Johor		DAWSON 1985
	Seagrass	Lahad Datu		OBIS
		Merambong	*	

Table 5.1 continued

Species	Habitat	Locality	Present Study	Others
Hippochthys heptagonus Bleerker, 1849 ^{LC}	Seagrass	Merambong	*	
Hippichthys penicillus (Cantor, 1849) ^{NE}	Rivers	Penang		KUITER 2009
	Estuary	Morib		BMNH
	Seagrass	Setiu	*	
Hippichthys spicifer (Rüppell, 1838) ^{LC}	Estuary	Tanjung Tuan		FOWLER 1938
Ichthyocampus carce (Hamilton, 1822)^{NE}	Rivers	Perepat River		UBC
	Stream	Kuala Sepetang	*	
	Estuary	Kota Tinggi		FMNH
		Muar		FOWLER 1938
Micrognathus andersonii (Bleeker, 1858)^{NE}	Seagrass	Perhentian / Redang	*	
	Macroalgae	Semporna Islands		KUITER 2009
	Coastal / Shallow waters	Tunku Abdul Rahman Park		DAWSON 1985
Micrognathus brevirostris brevirostris (Rüppell, 1838)^{NE}	Macroalgae	Malaysia		BMNH
Micrognathus micronotopterus (Fowler, 1938)^{NE}	Coastal / Shallow waters	Tunku Abdul Rahman Park		DAWSON 1985
Micrognathus natans Dawson, 1982^{NE}	Estuary	Kuala Bernam		OZCAM
Microphis brachyurus brachyurus (Bleeker, 1853)^{NE}	River Stream Estuary	Tawau		USNM; CAS; DAWSON 1985
Microphis manadensis (Bleeker, 1853)^{NE}	River Stream	Sandakan		DAWSON 1985

Table 5.1 continued

Species	Habitat	Locality	Present Study	Others
Notiocampus ruber (Ramsay and Ogibly, 1886)^{NE}	Coastal / Shallow waters Deep waters	Semporna Islands	AS	
Phoxocampus belcheri (Kaup, 1856)^{NE}	Coastal / Shallow waters	Mersing Islands		BMNH; DAWSON 1985
		Sandakan		DAWSON 1985
		Malaysia		FISHWISE 2007
Phoxocampus diacanthus (Schultz, 1943)^{NE}	Coastal / Shallow waters	Banggi Islands		USNM
Siokunichthys bentuviai Clark, 1966^{NE}	Seagrass Coastal / Shallow waters	Pulai River		CHOO et al. 2009
Siokunichthys nigrolineatus Dawson, 1983 ^{LC}	*Fungia* sp.	Perhentian / Redang	*	
		Tenggol Island	*	
		Semporna Islands	*	
		Malaysia		KPM
Syngnathoides biaculeatus (Bloch, 1785) ^{DD}	Macroalgae Seagrass	Penang		FOWLER 1938
		Pulai River	*	CHOO et al. 2009
		Perhentian / Redang	*	
		Malacca		BMNH
		West Malaysia		USNM
		Semporna Islands	*	
		Sugud Islands		RG
		Tunku Abdul Rahman Park	*	CAS
		Banggi Islands	*	SU
		Sandakan	*	
Syngnathus pelagicus Linnaeus, 1758^{NE}	*Sargassum* Macroalgae	Carey Island		MNHN

Table 5.1 continued

Species	Habitat	Locality	Present Study	Others
Trachyrhamphus bicoarctatus (Bleeker, 1857)$^{\mathrm{NE}}$	Macroalgae	Kapas Island	*	
	Seagrass	Perhentian/ Redang	*	
	Coastal / Shallow waters	Payar/ Langkawi	*	
		Tenggol Island	*	
		Mersing Islands	*	
		Sugud Islands		RG
		Johor		DAWSON 1985
		Semporna Islands	*	ALLEN 1992
		Sarawak	*	
		Tunku Abdul Rahman Park		
Trachyrhamphus longirostris Kaup, 1856$^{\mathrm{NE}}$	Macroalgae	Perhentian/ Redang	*	
	Seagrass	Kapas Island	*	
	Coastal / Shallow waters	Semporna Islands	*	
		Tunku Abdul Rahman Park		FISHWISE 2007
Trachyrhamphus serratus (Temminck and Schegel, 1850)$^{\mathrm{NE}}$	Coastal / Shallow waters	Johor	*	

Twenty-one species are restricted to Sabah and eleven to Peninsular Malaysia. Thirteen species are recorded for the first time, while two species from anecdotal records have yet to be confirmed (Table 3.2). Two species, *Siokunichthys bentuviai* Clark, 1966 and *Syngnathus pelagicus* Linnaeus, 1758 are species previously not believed to be distributed within the Malaysian region.

5.1.2 Fish habitat

The habitats of syngnathid fishes in Malaysia encompass fresh waters, brackish waters and marine waters, with six, four and 46 species respectively. Most syngnathids are found in shallow inshore habitats such as estuaries, coral reefs, macroalgal and seagrass beds (Figure 5.1), with only a limited number of species recorded from rivers, streams and deep sea habitats. Syngnathinae is the only subfamily with members dwelling in fresh waters, while Solegnathinae has only species dwelling in deep water habitats. A high degree of habitat specialization is observed in this study; several species of syngnathid are found to be habitat specific, being restricted to particular habitats, microhabitats or organisms (Table 5.3). All freshwater syngnathids in Malaysia are highly habitat specific, most dwelling among freshwater weeds in the pool zone of rivers. *Doryichthys boaja* is, however, found in the brackish waters of the Pulai River. Several marine syngnathids also display habitat specificity such as all the pygmy seahorses and *Siokunichthys negrolineatus*. Pygmy seahorses are found to live on gorgonian seafans which serve as their host, for instance, *Hippocampus bargibanti* was found on *Muricella* seafans. *Siokunichthys negrolineatus* was observed to live among the tentacles of the solitary mushroom coral, *Fungia* sp. in the Semporna Islands.

Table 5.2: First records of syngnathid species and species with restricted distribution in Malaysia

Species with very restricted distribution		First records	Anecdotal records
Peninsular Malaysia	Sabah		
Solegnathus hardwickii	*Doryrhamphus negrosensis*	*Doryrhamphus janssi*	*Notiocampus ruber*
Hippichthys penicillus	*Dunckerocampus pessuliferus*	*Hippocampus bargibanti*	
Hippichthys spicifer	*Hippocampus barbouri*	*Hippocampus denise*	
Hippichthys heptogonus	*Hippocampus bargibanti*	*Hippocampus mohnikei*	
Ichthyocampus carce	*Hippocampus denise*	*Corythoichthys amplexus*	
Micrognathus natans	*Hippocampus satomiae*	*Corythoichthys flavofasciatus*	
Siokunichthys bentuviai	*Hippocampus severnsi*	*Halicampus grayi*	
Syngnathus pelagicus	*Solegnathus lettiensis*	*Halicampus macrorhynchus*	
Hippocampus mohnikei	*Choeroichthys brachysoma*	*Hippichthys heptogonus*	
Acentronura tentaculata	*Corythoichthys intestinalis*	*Acentronura tentaculata*	
Trachyrhamphus serratus	*Corythoichthys ocellatus*	*Solegnathus hardwickii*	
	Corythoichthys schultzi	*Trachysrhamphus longirostris*	
	Cosmocampus banneri	*Trachyrhamphus serratus*	
	Festucalex erythraeus		
	Halicampus brocki		
	Halicampus grayi		
	Mictognathus micronotopterus		
	Microphis brachyurus brachyurus		
	Mictophis manadensis		
	Phoxocampus diacanthus		

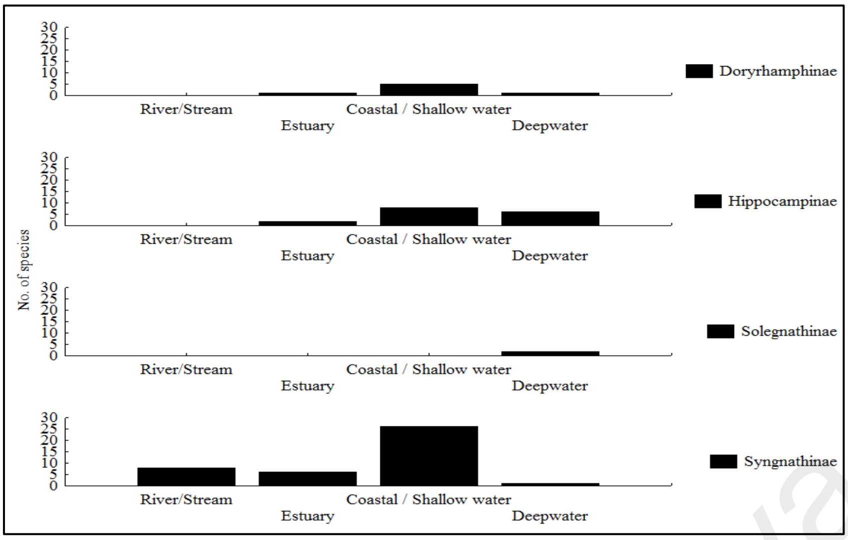

Figure 5.1: **Frequency distribution of Malaysian syngnathid species by habitat and subfamily.**

5.1.3 Threats to syngnathids

The most frequent threats to these fishes are exploitation (42.8%), by-catch (32.1%), habitat destructions and degradation (23.2%), recreational activities (8.9%) and pollution (18.0%) (Table 5.3). However, 15 species or 26.8% of Malaysian syngnathids remain unevaluated for threats due to lack of information. Exploitation of syngnathids in Malaysia is mostly associated with TCM trade, aquarium and curio trade while habitat destruction and degradation refers to destructive fishery and development. All freshwater dwelling species are listed as being threatened by pollution.

5.2 Discussion

5.2.1 Syngnathid taxonomy problems

The classification of syngnathid species especially seahorses remains contentious due to their external morphology that lacks the distinguishing characters normally found in other bony fishes (Lourie *et al*., 2004). Several recent additions

Table 5.3: Numbers and habitats of Malaysian syngnathids collected in the field. RV = river and stream, ES = estuary, MG = mangrove, SG = seagrass meadow, SW= seaweed bed, CR= coral reef, SSC= specific scleractinian coral, SC = soft coral, SB = sandy bottom, shallow waters, and DW = deep waters (>30m). Numerals indicate number of specimens collected. NS= number of surveyed sites; total number of sites surveyed = 35 (see Figure 2.1 for more details).

Species / Threat	NS	RV	ES	MG	SG	SW	CR	SSC	SC	SB	DW	Further Remarks
Acentronura tentaculata	15				15							
Dunckerocampus pessuliferus	1						1					Reef flat
Dunckerocampus dactyliophorus	4						6					Reef flat
Doryrhamphus excisus excises	2						2					Reef flat
Doryrhamphus janssi	4						4					Reef flat
Hippocampus barbouri	3					12						*Halimeda* sp.
Hippocampus bargibanti	2								4			*Muricella* sp.
Hippocampus comes	6				1		12					Reef crest
Hippocampus denise	2							3				
Hippocampus histrix	3					2						*Halimeda* sp.
Hippocampus kelloggi	6										17	
Hippocampus kuda	7		2	2	68							*Enhalus acoroides*
Hippocampus mohnikei	5		4		1							
Hippocampus satomiae	1								1			
Hippocampus severnsi	1								1			
Hippocampus spinosissimus	15								4	23	313	Sea whips
Hippocampus trimaculatus	19									19	305	
Corythoichthys amplexus	4						3					Reef flat
Corythoichthys flavofasciatus	3						2					Reef flat
Corythoichthys haematopterus	2						6					Reef flat

Table 5.3 continued

Species / Threat	NS	RV	ES	MG	SG	SW	CR	SSC	SC	SB	DW	Further Remarks
Corythoichthys intestinalis	1						2					Reef flat
Doryichthys boaja	2	3	1									Freshwater weeds
Doryichthys deokhatoides	2	4										Freshwater weeds
Doryichthys martensii	1	2										Freshwater weeds
Halicampus brocki	1						1					Coral rubble
Halicampus grayi	1		3				5					Silt-muddy bottom
Halicampus macrorhynchus	2						2					Coral rubble
Hippichthys heptagonus	1				1							
Hippichthys penicillus	1			1								
Hippichthys cyanospilos	2		2		4							
Ichthyocampus carce	2		1	3								Muddy tidepools
Siokunichthys nigrolineatus	3							2				*Fungia* sp.
Siokunichthys bentuviai	1				1							*Enhalus acoroides*
Syngnathoides biaculeatus	6				14							*Enhalus acoroides*
Trachyrhamphus bicoarctatus	7				1		2		2	5		
Trachyrhamphus longirostris	3								2			
Trachyrhamphus serratus	3									3		
Total:		**9**	**13**	**6**	**106**	**3**	**47**	**5**	**11**	**52**	**635**	

Table 5.4 **Threats to Malaysian syngnathids. Threat type (EX = Exploitation; BC = By-catch; HD = Habitat destruction and degradation; REC = Recreational activities and POL = Pollution).**

Species / Threat	EX	BC	HD	REC	POL
Dunckerocampus pessuliferus	*				
Dunckerocampus dactyliophorus	*				
Doryrhamphus excisus excisus	*				
Doryrhamphus janssi	*				
Doryrhamphus negrosensis	*				
Hippocampus barbouri	*	*	*		
Hippocampus bargibanti				*	
Hippocampus comes	*	*	*		
Hippocampus denise				*	
Hippocampus histrix	*	*			
Hippocampus kelloggi	*	*			
Hippocampus kuda	*	*	*		*
Hippocampus mohnikei	*				
Hippocampus satomiae				*	
Hippocampus severnsi				*	
Hippocampus spinosissimus	*	*	*		
Hippocampus trimaculatus	*	*	*		
Kyonemichthys rumengani					
Solegnathus hardwickii	*	*			
Solegnathus lettiensis	*	*			
Choeroichthys brachysoma					
Corythoichthys amplexus	*				
Corythoichthys flavofasciatus					
Corythoichthys haematopterus	*				
Corythoichthys intestinalis	*				
Corythoichthys ocellatus	*				
Corythoichthys schultzi	*				
Cosmocampus banneri					
Doryichthys boaja	*		*		*
Doryichthys deokhatoides	*		*		*
Doryichthys martensii	*		*		*
Festucalex erythraeus					
Halicampus brocki					
Halicampus grayi		*			
Halicampus macrorhynchus					
Hippichthys heptagonus			*		
Hippichthys penicillus		*			
Hippichthys cyanospilos		*			
Hippichthys spicifer		*			
Ichthyocampus carce			*		*
Micrognathus andersonii					
Micrognathus brevirostris brevirostris					
Micrognathus micronotopterus					
Micrognathus natans					

Table 5.4 continued

Species / Threat	EX	BC	HD	REC	POL
Microphis brachyurus brachyurus					*
Microphis manadensis					*
Phoxocampus belcheri					
Phoxocampus diacanthus					
Siokunichthys nigrolineatus					
Siokunichthys bentuviai			*		*
Syngnathoides biaculeatus	*	*	*		*
Syngnathus pelagicus					
Trachyrhamphus bicoarctatus		*			
Trachyrhamphus longirostris		*			
Trachyrhamphus serratus		*			

suggest that there may be more new species from Australia, although not all have been widely accepted as new species (see Teske *et al.*, 2007; Scales, 2010). Kuiter's (2009) recent pictorial guide book on syngnathids is the most comprehensive collation to date, but lacks formal taxonomic descriptions and a number of previously removed synonyms of seahorses have been reintroduced without justification.

5.2.2 Diversity and distribution

Syngnathids in Malaysian waters are largely unknown with very limited research and records available. Hitherto, the only comprehensive records on the diversity and distribution of syngnathids are those pertaining to seahorses (Hippocampinae) in Peninsular Malaysia (Choo and Liew, 2003) and East Malaysia (Choo and Liew, 2004), whereas records on the other subfamilies are scattered in various publications. The present study shows that Malaysia is diverse in syngnathids with 56 species in 19 genera, representing 34.5% and 18.9% of the world's known syngnathid genera and species respectively. This richness however, could not match that of Australian waters which contain 36% of the world's described species, 25% of which are endemic species (Martin-smith and Vincent, 2006, Kuiter, 2009). Nearly 73% of the Malaysian syngnathid species are found in Sabah, which is part of the 'Coral Triangle', believed to be the Earth's richest marine biodiversity region (Allen and Werner, 2002). Due to their cryptic nature, small size and generally low abundance, syngnathids are likely to be under represented in most collections, particularly catches from large fishing gears (Martin-smith, 2008). Therefore, new records of syngnathid in Malaysia are expected to be reported over time. Syngnathids of Malaysia are poorly recorded; that recorded lacked information of their diversity and distribution with the exception of seahorses (Hippocampinae) which have been comprehensively recorded by Choo and Liew (2003; 2004). The present study has added thirteen unreported occurrences and one anecdotal records (see Table 3.2) thus confirming that the number of Malaysian species was severely under estimated. The recorded species from anecdotal sources (Table 5.2) however, requires further research to confirm their existence.

Seven species from Hippocampinae have been previously recorded in Malaysia (Choo and Liew, 2004). The present study updates the existing record to thirteen Hippocampinae species, with the additions of *Hippocampus bargibanti* Whitley, 1970; *Hippocampus denise* Lourie and Randall, 2003; *Hippocampus mohnikei* Temminck and Schegel, 1850; *Hippocampus satomiae* Lourie and Kuiter, 2008; *Hippocampus severnsi* Lourie and Kuiter, 2008; and *Kyonemichthys rumengani* Gomon, 2009. Twelve confirmed species are reported in Sabah, whereas seven species are now found in Peninsular Malaysia with the addition of *Hippocampus comes* Cantor, 1850; *Hippocampus mohnikei* Temminck and Schegel, 1850; and *Hippocampus histrix* Kaup, 1856 which were not previously reported by Choo and Liew (2003). *Hippocampus comes* is now believed to be more ubiquitous in distribution within Peninsular Malaysia than previously thought.

The present study also sheds light on *Syngnathus pelagicus*, which until now is believed to be absent from the Malaysian region. It is recorded from Carey Island (Selangor) in peninsular Malaysia. The discovery of *Siokunichthys bentuviai* in the Pulai River, Johor is another interesting discovery as this was a first in Malaysia and second in the region after Kuiter (2009) reported it in Indonesia. This species was documented as endemic to the Red Sea (Dawson, 1985).

The distribution of *Syngnathus pelagicus* within the Indo-Pacific region needs confirmation as existing documentation does not support it. The nearest record of its distribution is in Hong Kong (Sadovy and Cornish, 2000), but major records suggest that this species likely originated from the Atlantic Ocean. Although the lone specimen recorded off Carey Island suggests the possibility of rafting on drifting seaweed (Teske *et al*., 2005) or somehow transported by a ship since the site is located near Port Klang, Selangor, there is no real evidence of it. Nonetheless, the possibility of misidentification cannot be ruled out.

Notiocampus ruber, (Ramsay and Ogibly, 1886) sighted around the Semporna Islands, is listed as anecdotal record since there was only one photo evidence, and since then, none has been reported despite the intensive diving activities around the area. *Notiocampus ruber* is a cryptic species, rare and believed to be endemic to Australian waters (Baker *et al*., 2008).

Syngnathid fishes are recorded over a broad geographical area; however, the actual range of these species is largely restricted to suitable habitats determined by various factors such as body size, camouflage ability, food and reproduction (Foster and Vincent, 2004). Most habitats harbour a low abundance of syngnathid fishes with the exception of seagrass meadows where they are generally considered the dominant fish taxa in terms of number (Martin-Smith, 2008). Seahorses in particular are most commonly reported in seagrass, algal and least common in mangrove areas (Foster and Vincent, 2004). There are other species of syngnathid fishes that show special preferences for using a specific species of plant and animal as holdfast, such as *Hippocampus bargibanti* (Lourie and Randall, 2003) and *Siokunichthys nigrolineatus* (Phillips and Pullin, 1987). Sabah has a complex coastal geomorphology (Morgan and Valencia, 1983), and thus offers a wide range of habitat types; this could explain the richness of syngnathids in Sabah as compared to other parts of Malaysia. However, it is noted that Sabah also offers the most number of attractive coral reefs and diving spots, and hence the higher diversity of syngnathids could be due to the greater search effort.

Syngnathids that lived in very specific or localized habitats such as *Hippocampus bargibanti* and *Hippocampus denise* which live on gorgonian seafans (Reijnan *et al*., 2011), face higher extinction risk. The number of species living in highly localised habitats in Malaysia is highest in the Semporna Islands. These syngnathids are seen as the most vulnerable to environmental changes caused by pollution, habitat destruction and human disturbance (Chong *et al*., 2010).

5.2.3 Exploitation

Syngnathids are extremely susceptible to anthropogenic threats because they occur in low densities, possess low mobility and are extremely site faithful (Vincent, 1995; Perante *et al*., 2002). Efforts to control and minimize the exploitation of syngnathid fishes in Malaysia remain a challenge since trawling, a a common fishing method is primarily responsible for seahorse by-catch (Choo and Liew, 2005). Although syngnathids do not constitute a targeted commercial species, their incidental catches by trawling in open waters are not discarded but kept and traded. Shrimp trawls operating in shallow waters catch most of the common seahorses (*Hippocampus trimaculatus*, Leach, 1814 and *Hippocampus kuda*, Bleeker, 1852) (Choo and Liew, 2003). A targeted syngnathid fishery, however, does exist among traditional fishermen who use seine nets, scoop nets and bottom drift nets to fish them in coral reefs, seagrass beds, lagoons, and rivers; syngnathid catches supplement their low income (Choo and Liew, 2005).

The current overexploitation of syngnathid fishes is largely due to the market demand for Traditional Chinese Medicine (TCM) or Traditional Medicine (TM) market, curios and the live aquarium trade. Species traded as TM and TCM are mainly seahorses (*Hippocampus* spp.), pipehorses (*Solegnathus* spp.) and the Alligator pipefish (*Syngnathoides biaculeatus*, (Bloch, 1785)) (Martin-Smith *et al*., 2003), with pipehorses believed to be highest in medicinal value and the most valuable for the trade (Vincent, 1996). Malaysia is touted to be an important supplier of pipefish, pipehorse (Martin-Smith *et al*., 2003) and seahorse (Perry *et al*., 2010). The fish are primarily exported to other Asian countries such as Hong Kong and Taiwan with a maximum annual trade volume of 1280 kg year^{-1}for seahorses (Perry *et al*., 2010) and 683 kg year^{-1} for both pipefish and pipehorse in 2001 (Martin-Smith *et al*., 2003). However, decline in catch rates are already reported by local fishers (Perry *et al*., 2010).

5.2.4 Habitat and environmental degradation

Habitat destruction of important syngnathid habitats, such as seagrass beds, poses a major cause for concern. The seagrass beds in the Pulai River Estuary, Johor, is a significant habitat for *Hippocampus kuda, Hippocampus comes*, *Syngnathoides biaculeatus* and *Siokunichthys bentuviai* as well as other endangered marine mammals and reptiles (Choo *et al*., 2009) presently threatened by habitat loss and degradation due to industrial development.

Destructive fishing methods were vastly employed in the 1990's and remains the main cause of fish decline in Sabah. Both blast and cyanide fishing methods have ruined formerly pristine reefs of islands off Semporna (Burke *et al*., 2002). Although, blast fishing within protected reefs has been largely controlled since the establishment of marine parks (Pilcher and Cabanban, 2000), this and other destructive fishing techniques are still being employed by illegal fishing vessels, particularly around the borders of Malaysian waters.

Another threat to syngnathid populations of Malaysia is the increasing problems of aquatic pollution. Aquatic pollution is mainly from land based sources, particularly in the west coast of Peninsular Malaysia which has the highest human concentration and high-traffic shipping lanes along the Straits of Malacca (Burke *et al*., 2002). Several rivers of Malaysia, including the Pulai and Johor Rivers are considered degraded ecosystems with pollution being one of the major factors affecting them (Chong *et al*., 2010). The once pristine Gombak River, Selangor was reported to be home to *Doryichthys deokhatoides,* (Bleeker, 1853) in 1969 (Bishop, 1973), but the fish had since disappeared from the river when it was surveyed in 1985 and again in 1990. Its disappearance is presumably due to land development and pollution as the city of Kuala Lumpur expanded (Zakaria-Ismail, 1994).

5.2.5 Conservation and legislation

Currently 71 species of syngnathids are listed in the 2010 IUCN Red List (IUCN 2010). As a result of their continuing decline and trade threat, the *Hippocampus* species are now also listed in CITES Appendix II (CITES 2002). Such listing, however, does little to protect them from intensive local collections. Despite their high vulnerability, syngnathids like other rare or habitat restricted fishes are not mentioned in the federal

Fisheries Act 1985 [Act 317], unlike special protection accorded to aquatic mammals and turtles. Notwithstanding, the Fisheries Act does not protect aquatic species, and freshwater and marine resources may be adversely affected by land-based activities which are essentially managed by state governments and their respective agencies. Herein, lies the problem of sectoral-based management and the implementation of effective fishery management, since state jurisdiction may overlap and be in conflict with federal jurisdiction. Given this scenario and the increasing anthropogenic threats to the Malaysian ichthyofauna (see Chong *et al.*, 2010), integrated coastal or ecosystem-based management seems more urgent than ever to help conserve the Malaysian syngnathids, and fisheries resources in general. At present in Malaysia, the designated Marine Protected Area (MPA) probably offers the best protection for syngnathids from most ongoing threats. However, the MPAs are not large enough and only protect fringing areas two nautical miles from the lowest low tide at shoreline (DMPM, 2010). No equivalent protected areas are however designated in fresh waters and brackish waters with the exception of aquatic zones within national reserves.

Syngnathid exploitation and management in the wider context of community-based fishery management may be implemented in certain areas, such as near coral reefs. Community-based seahorse management as exemplified in the Philippines has shown that grassroot conservation effort can be strongly fostered in areas where seahorse is the target fishery (Martin-Smith *et al.*, 2004). Unlike other fishes, the charismatic seahorses can garner public support and action for their conservation. Save Our Seahorses (S.O.S.) Malaysia, a local non-governmental organisation has been quite successful in using seahorses as flagship species in its conservation programs to promote not only the protection of seahorses but also their habitats (Choo 2007).

In summary, a comprehensive documentation of the fish diversity of the family Syngnathidae in Malaysia was achieved in this chapter. The results revealed that Malaysia can be classified with high species richness of this family of fishes that encompasses all subfamilies of the Syngnathidae. The Semporna region in the state of Sabah has been identified as the area recorded with the highest species richness throughout the country.

CHAPTER 6: SYNTHESIS AND GENERAL DISCUSSION

The synthesis here proposes and tests the following hypotheses: -

1) Acoustic signature of syngnathids is related to their habitat type or environment. (6.1)
2) The sound producing mechanism associated with click in syngnathids has evolved from the ancestral form, the three-cranial bone model; all present day syngnathids have either retained or modified this model. (6.3)

6.1 Syngnathid habitat preferences and acoustic signatures.

Malaysia is blessed with a high species richness of syngnathid fishes occupying aquatic habitats (see Chapter 5) and the identification of syngnathid habitat preference has allowed this study to examine the possible relationship between sound production and habitat selection. Habitat selection by syngnathid fishes is an important aspect in their behavioural ecology due to their small home ranges and slow swimming nature (Curtis and Vincent, 2005). While the important roles of various aquatic habitats to syngnathid fishes have been well documented (refuge and greater food source), the relationship between habitat selection and fish bioacoustics generally remains poor (Kendrick and Hyndes, 2003).

Is it possible that the acoustical properties and structural mechanisms of sound producing capabilities in syngnathid fishes are influenced by habitat preference? Different aquatic habitats have been known to emit habitat specific ambient noise with significantly different intensities bearing cues of habitat quality (Radford *et al*, 2010; Bertucci *et al*., 2015). While it has been reported that coral and fish larvae use reef sounds as cues for orientation prior to settlement (Simpson *et al*., 2004; Vermeij *et al*., 2010), very few studies were made on how settled fishes interact or adapt to their habitat's ambient noise. Different habitats have been known to pose different ecological constraints that affect the production, transmission and reception of acoustic signals (Lugli, 2015). Environmental factors such as water depth, acoustical complexity, ambient noise, presence of reflecting surfaces and salinity have been found to affect acoustic signals (Wiley and Richards, 1982; Hawkins and Myrberg, 1983; Roger and Cox, 1988; Mann and Lobel, 1997; Patten, *et al*, 2004; Au and Hastings, 2008; Fine and Parmentier, 2015; Lugli, 2015).

While in this study we did not directly assess the effects of these factors (i.e. habitat environmental factors) on the acoustic signals generated by the sampled syngnathid fishes, an attempt was made to examine possible correlations of syngnathid habitat preference with the parameters of the acoustic signal they produce. Aquatic habitats were ranked based on four factors (depth, salinity, complexity and presence of hard surfaces), while signal characteristics of syngnathid feeding clicks were based on previous results (Chapter 3 and 4) along with other similar studies (Table 6.1). The canonical correspondence analysis (CCA) triplot result for syngnathid habitat preferences reveals that the various species are well demarcated according to their habitat preference (Figure 6.1). Thus, *Hippocampus trimaculatus*, *H. barbouri*, *H. spinosissimus*, and *Trachyrhamphus* are those species that prefer deep marine water in habitats of sandy bottoms (SD) or where sponges and octocorals (SP) and seaweeds (SW) are found, while *Syngnathoides biaculeatus* (Sb), *Acentronura tentaculata* (At), *Hippocampus kuda* (Hk), *H. comes* (Hc), *Corythoichthys haematopterus* (Ch) and *Dunckerocampus dactylipohorus* (Dund) are species that prefer structurally-complex biotopes in shallow marine waters such as coral reefs (CR), seagrass beds (SG) and mangroves (MG). Species that are found in freshwater habitats include *Doryichtys boaja* (Db), *D. martensii* (Dm) and *D. deokhathoides* (Dd). The biplot result from the principal component analysis (PCA) of feeding click signal parameters (Figure 6.2) however did not show any relationship between the measured click parameters and species, suggesting a general overlap of the values of these parameters. There are also no obvious differences in the signal parameters produced by the three-bone sound mechanism; except that the modified two-bone type tends to have longer signal duration except in *H. barbouri* (Hb). There is no observable relationship between species habitat preference and feeding click signal parameters in Seahorses (*Hippocampus* spp.), despite a morphologically similar sound producing mechanism within the genus. Similarly, pipefishes also did not display any noticeable grouping. Thus, the habitat preference of syngnathid fish species appears independent of the morphological arrangements and specialization of the sound producing mechanism.

Pipefishes were observed to be produce lower minimum frequency feeding sounds compared to seahorses. The freshwater pipefishes, *D. martensii* (Dm) and *D. deokhahtoides* (Dd) were found to be placed fairly distant from one another, the former having higher frequency and the latter with shorter duration of the signal (Figure 6.2). This is likely to be attributed to the different forms of the three-bone mechanism revealed in Chapter 4. In comparison, both *Syngnathus* pipefishes (by Ripley and Foran, 2007) were placed close to each other (Sf, Sr), possibly as a result of the same mechanism. It would be interesting to examine the sound producing mechanism and feeding click signal parameters of pipefishes from the genus *Syngnathus* because it is a large genus with 32 described species, mostly associated with macroalgal habitats and is believed to be one of the more primitive and least changed from the ancestral form (Dawson, 1985; Kuiter, 2009).

Table 6.1: Sound parameters of syngnathid feeding clicks and its habitat.

Species (code)	Habitat	Frequency (kHz)		Duration (ms)		Study
		minimum	maximum	minimum	maximum	
Hippocampus comes (Hc)	Coral reef; Seaweed	1.04	1.25	4.15	34.51	Present study
Hippocampus trimaculatus (Ht)	Muddy / Sandy bottom	1.03	2.32	3.90	29.70	Present study
Hippocampus barbouri (Hb)	Coral reef; Seagrass	1.39	3.98	5.40	11.25	Present study
Hippocampus kuda (Hk)	Mangrove; Seagrass; Coral reef	1.00	4.80	4.00	20.00	Chakraborty *et al.*, 2015
Hippocampus zosterae (Hz)	Seagrass	2.65	3.43	5.00	20.00	Colson *et al.*, 1998
Hippocampus erectus (He)	Seagrass; Mangrove; Sponges	1.96	2.37	4.00	20.00	Colson *et al.*, 1998
Hippocampus reidi (Hr)	Mangrove; Seagrass; Coral reef	0.80	1.35	7.2	27.1	Oliveira *et al.*, 2015
Doryichthys martensii (Dm)	Freshwater river and streams	0.51	1.12	4.26	11.56	Present study
Doryichthys deokhatoides (Dd)	Freshwater river and streams	0.66	4.00	2.35	5.33	Present study
Syngnathus fuscus (Sf)	Seagrass; Sandy bottom	1.30	1.45	7.00	17.00	Ripley and Foran, 2007
Syngnathus floridae (Sr)	Seagrass	0.98	1.45	5.00	22.00	Ripley and Foran, 2007
Syngnathoides biaculeatus (Sb)	Seagrass	2.31	5.44	2.53	9.39	Present study

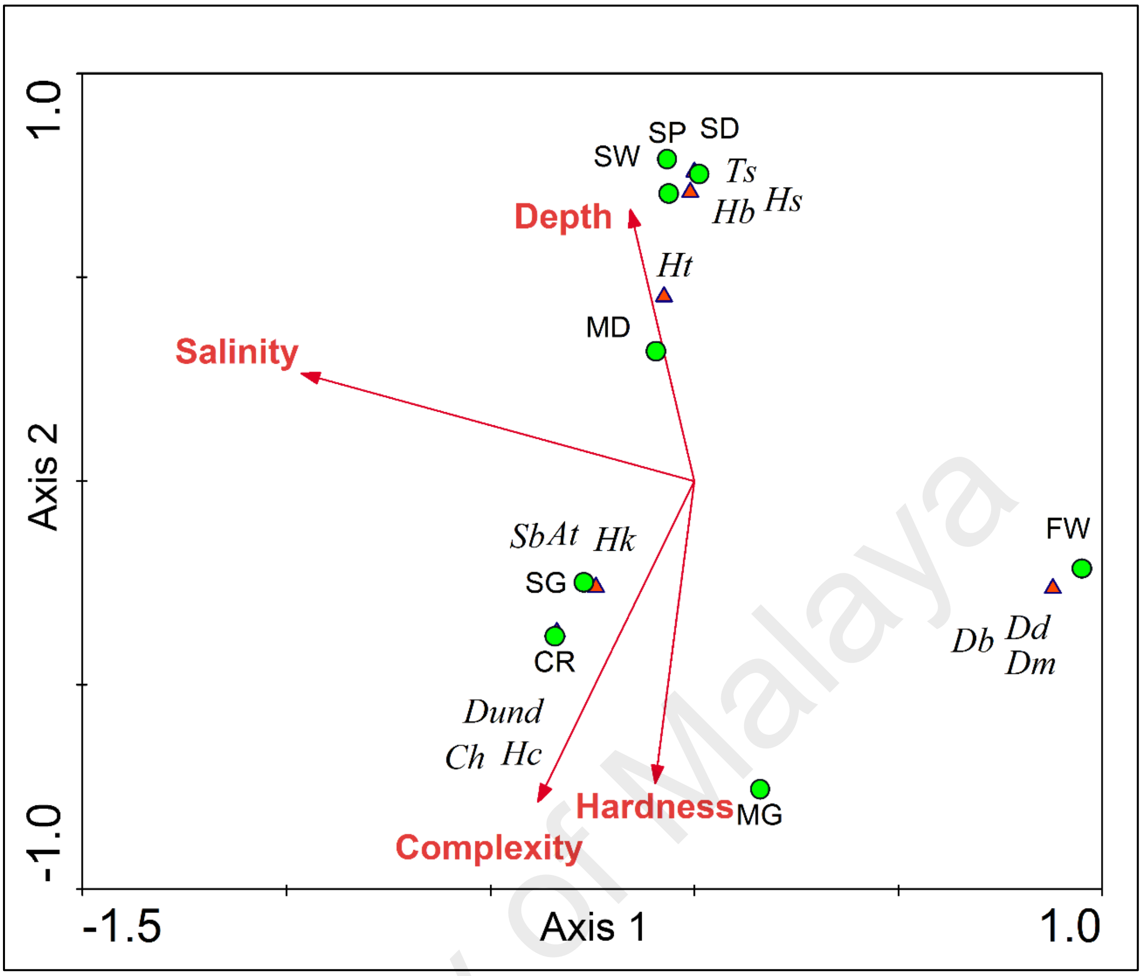

Figure 6.1: Canonical correspondance analysis (CCA) of syngnathid habitat preferences (Hardness= presence of hard surfaces; Complexity; Depth and Salinity) (CR = Coral Reef; FW = Freshwater; MG = Mangrove; SD = Sandy bottom; MD = Muddy bottom; SG = Seagrass; SP = Sponges and Octacorals; SW = Seaweed) (Species: *At* = *Acentronura* tentaculata; Ch = *Corythoichthys haematopterus*; *Db* = *Doryichthys boaja*; *Dd* = *Doryichthys deokhahtoides*; *Dm* = *Doryichthys martensii*; *Dund* = *Duncekerocampus dactyliophorus*; *Hb* = *Hippocampus barbouri*; *Hc* = *Hippocampus comes*; *Hk* = *Hippocampus kuda*; *Hs* = *Hippocampus spinosissimus*; *Ht* = *Hippocampus trimaculatus*; *Hz* = *Hippocampus zosterae*; *Sb* = *Syngnathoides biaculeatus*; and *Ts* = *Trachryrhamphus bicoarctatus*)

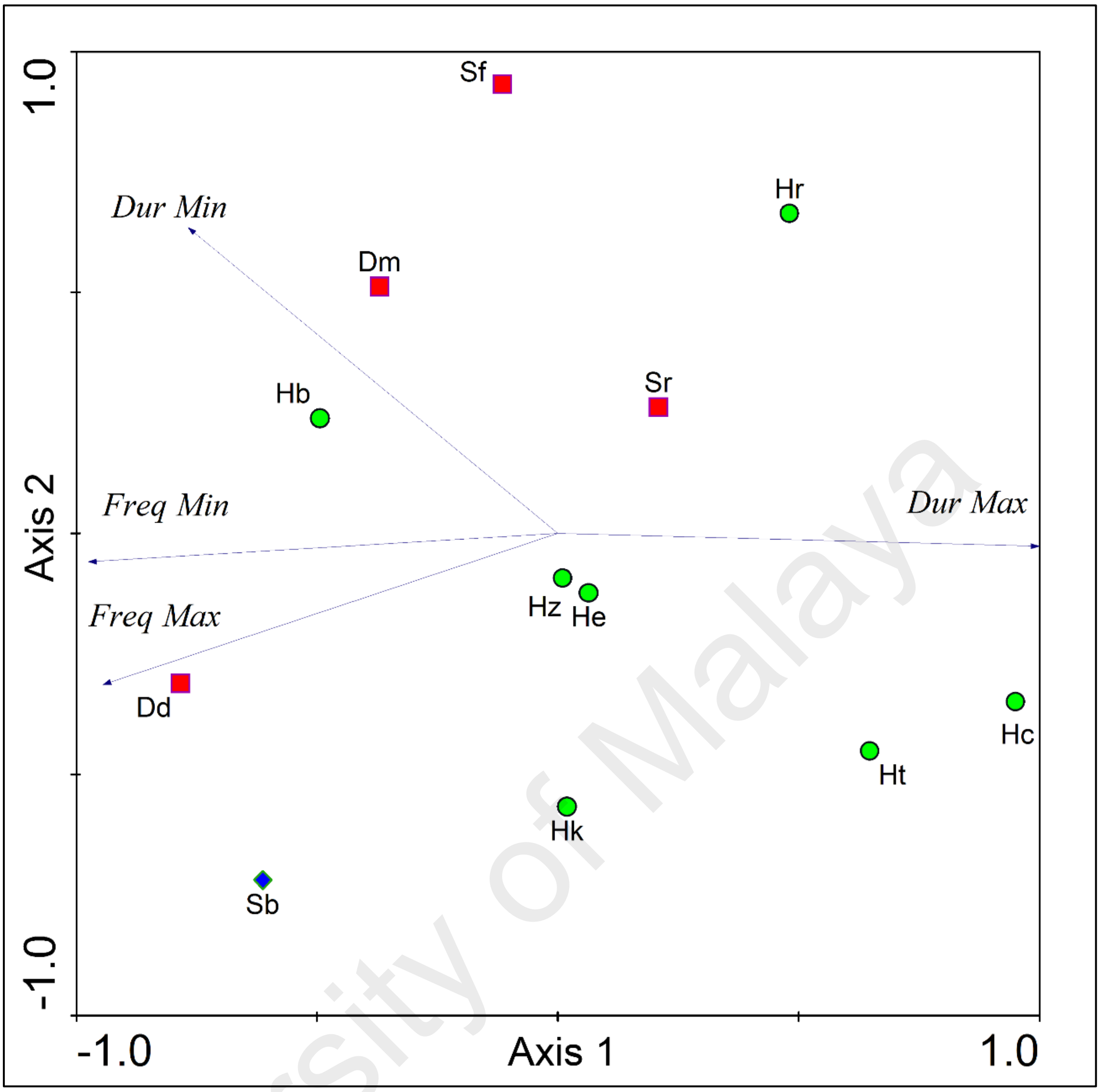

Figure 6.2: Principal component analysis (PCA) of feeding click signal (DurMax = Maximum signal duration; Durmin = Minimum signal duration; FreqMax = Maximum peak frequency and FreqMin = Minimum peak frequency) of 12 species of syngnathid species. Species symbols are denoted by its sound producing mechanism (Diamond: 2 bones = supraoccipital-postcranial 2; Square: 3 bones = supraoccipital-postcranial 1-postcranial 2; and Circle: Modifed 2 bones = supraoccipital-coronet). (Species: Dd = *Doryichthys deokhahtoides*; Dm = *Doryichthys martensii*; Hb = *Hippocampus barbouri*; Hc = *Hippocampus comes*; He = *Hippocampus erectus*; Hk = *Hippocampus kuda*; Hr = *Hippocampus reidi*; Ht = *Hippocampus trimaculatus*; Hz = *Hippocampus zosterae*; Sb = *Syngnathoides biaculeatus*; Sf = *Syngnathus fuscus*; and Sr = *Syngnathus floridae*)

The Spearman rank order correlations test between habitat preference and feeding click signal parameters displayed no statistical correlation between them (p >0.05). This means that the differences in acoustic signals and the morphology of the sound producing mechanism is unlikely to be controlled by the species's habitat preference despite most species showing site fidelity. Mann and Lobel (1998) compared the sounds produced by male *Dascyllus albisella* (Hawaiian dascyllus), in two different locations (separated by 1000 kilometers) and discovered that only pulse duration was significantly different between sites. Additionally, differentiation in acoustic parameters through geographic variation was further demonstrated in the oyster toadfish (Fine, 1978), clownfish (Parmentier *et al*., 2005) and minnows (Philips and Johnston, 2008). However, little is known of the differences in acoustic parameters and sound producing mechanisms between individuals of the same species inhabiting different habitats.

In summary, the present study revealed no correlation between habitat preference of syngnathid fishes and diferences in the sound producing mechanisms and their associated acoustic parameters. Hence, this analysis does not support the stated hypothesis. While no relationship was observed based on the examined parameters and available species, verification of no support for the hypothesis could be pursued by more investigations of the acoustic signals and sound producing mechanism of other syngnathid species.

6.2 Syngnathid acoustic signatures and sound producing mechanisms

Despite the many reports of acoustic signals in different behavioural contexts (Colson *et al*., 1998; Ripley and Foran, 2008; Anderson, 2009; Anderson *et al*., 2011; Chakraborty *et al.,* 2014; Haris *et al*., 2014; Oliveira *et al*., 2014), most studies adopt the short Fourier Transform approach of analysing the recorded signals, with the exception of Chakraborty *et al*. (2013; probability density function) and Haris *et al*. (2014;

multifractal detrended fluctuation). In contrast, this dissertation documents the acoustic signatures of syngnathid fishes using the wavelet transformation approach which have interestingly led to the discovery of syngnathid clicks as a multicomponent signal (see Chapter 3 and 4).

The scalograms (in Chapter 3 and 4) revealed the presence of the multicomponent signal throughout the family which was not detected in previous studies (Dufossé, 1874; Gill, 1905; Fish, 1953; Fish and Mowbray, 1970; Bergert and Wainwright, 1977: Colson *et al*., 1998; Ripley and Foran, 2008; Anderson, 2009; Anderson *et al*., 2011; Chakraborty *et al.*, 2014; Haris *et al.*, 2014; Oliveira *et al*., 2014). These signals have been linked to the sound producing mechanisms and its kinematics in the production of click and purr sounds. The high resolution spectral output of the recorded signals has further revealed the species-specific acoustic signatures that can now clearly be documented in tandem with the sound parameters.

Despite reporting the presence of species-specific signatures, the functionality of the feeding clicks could not be established. A conceivable hypothesis is that feeding clicks are used as advertisement calls to notify sympatric species of their territory and species of food availability. Moreover, syngnathid clicks have also been observed in other behavioural contexts, such as courtship (Anderson, 2009; Oliveira *et al*., 2014), competition (Vincent, 1994) and when in duress (Ripley and Foran, 2007; Anderson, 2009; Anderson *et al*., 2011; Oliveira *et al*., 2014). While previous reports have attempted to provide possible explanations to the clicks' function (Anderson, 2009, Oliveira *et al*., 2014), no concrete evidence could be ascertained, largely because of its production in various behavioural contexts and is hypothesised to provide other functional roles such as mate location, mate quality, alarm calls and for courtship (Ripley and Foran, 2007; Anderson, 2009; Anderson *et al*., 2011; Oliveira *et al*., 2014). While feeding clicks were produced consistently after every full strike, it is unlikely to be merely a by-product of prey capture because it may increase predation risk (Colson *et al*., 1998; Oliveira *et al*., 2014). Therefore, the actual role of clicks in syngnathids remains unclear. It is worthwhile to investigate the behavioural implications of syngnathid clicks. These signals could be spectrally different which would provide further insights on its functional role.

By comparing the feeding click signal parameters between seahorses (Chapter 3) and pipefishes (Chapter 4), the Mann-Whitney test showed significant differences between low frequency (Z': -5.38; $p < 0.05$) and high (dominant) frequency components (Z': -4.72; $p < 0.05$). Conversely, the signal duration of both the low frequency (Z': -0.52) and dominant components (Z': -0.15) were not significantly different ($p > 0.05$) from one another. The feeding strike of all syngnathid fishes consistently displays extremely rapid head elevation and prey capture (Bergert and Wainwright, 1997; Colson *et al*., 1998; Roos *et al*., 2009; Leysen *et al.* 2011). Evident from previous studies and the work described in this dissertation, the range of feeding click durations was observed to overlap between species (Fish, 1953; Colson *et al*., 1998; Chakraborty *et al.,* 2015; Oliveira *et al.,* 2015). Differences in feeding click duration between both group (seahorses and pipefishes) were not significantly different ($p > 0.05$) from one another despite being produced by different sound producing mechanisms. As for feeding click frequencies, the detection of statistical differences (Chapter 3.1.1 and 4.1.1) in the output could very well be due to the morphological structure of the sound-producing mechanisms and bone shape and size variation of the different species which will be discussed in more detail in the following sections. Thus, it is highly likely that this variation also lead to the production of species-specific acoustic signals in syngnathid fishes.

Interspecific differences in the temporal pattern of the acoustic signals (feeding clicks and distress growls) may be useful in species discrimination, mate evaluation and mate location, especially among closely related members of a species (Myrberg *et al*., 1972; Crawford *et al.*, 1997; Malavasi *et al*., 2008; Colleye et al., 2011). Similarly, systematic comparisons and the functional role of acoustic properties in species recognition between closely related species of pomacentrids, mormyrids and gobiids have been reported in marine fishes (Crawford *et al.,*1997; Kihslinger and Klimley, 2002; Sullivan *et al*., 2002; Malavasi *et al*., 2008). The same is likely to be true for syngnathids based on the evidences so far.

6.2.1 Possible functional role of the coronet (= first postcranial plate)

An interesting hypothesis that can be derived from this study is the possible functional role of the coronet (COR) bone in seahorses. Here, we strongly suspect that the COR bone is responsible for the variation in species acoustic signals. Evident from our results in Chapter 3, the sound producing mechanism is consistent throughout the *Hippocampus* genus with variations in the COR bone shape and size among species. Similarly, the acoustic shape for seahorse feeding clicks was consistent but with distinct variations in signal parameters (frequency and duration) among seahorse species. The main notable differences in the sound producing mechanism structure that may contribute to the differences in feeding click signals is the COR bone because the shape and size of the supraoccipital (SOC) bone among seahorse species is qualitatively similar. To date, the measurements of the seahorse coronet height, type of inclination, number of spines and overall shape were used in the classification of a species (Lourie *et al*., 2004; Freret-Meurer *et al.,* 2013). The exact functionality of the COR in the production of clicks has never been investigated. The closest studies used muting surgeries through the removal of the ridge on the dorsal posterior region of the SOC (Colson *et al.*, 1998; Anderson, 2009).

The initial characters of sound are determined by the physical properties of the source (material, shape and size), as well as, the nature of the excitation (van den Doel and Pai, 1998). In seahorses, the SOC and COR bones are made of the same material; in addition, the shape and size of the SOC bones are also almost similar. Assuming the strength at which both the SOC and COR bones stridulate are similar for each strike (nature of excitation), this leaves the shape and size of the COR bone as the only determining factor responsible for the variation in feeding click signal parameters. Additionally, the coronet of seahorses is known to be individually distinct, which functions as a finger print in individual identification (unique patterns of cavities and elevations) (Freret-Meurer and Andreata, 2008; Freret-Meurer *et al.* 2013). Despite recording no differences of signal parameters among individuals of the same species (Chapter 3), a detailed study on the matter would be required to further confirm it. It is possible that the minor differences in the coronet shape results in individual-specific signals.

While the sound producing mechanism is consistent throughout the seahorse genus, pipefishes were observed to possess different mechanisms without the coronet bone (Chapter 4). Like the coronet in seahorses, the first postcranial bone (POC1) in pipefishes, also varies in shape and size with species. The shape and size of the SOC also greatly varies in pipefishes unlike that of seahorses. Although this study did not investigate the signal parameters of two or more pipefish species from the same type of sound producing mechanism (Chapter 5), it is presumed that pipefishes probably generate a larger variety of signals due to the variable shape of its SOC (Chapter 5). Thus, it is unlikely that the acoustic signal remains the same even for pipefishes that have the same sound generating mechanism. It would be interesting to also examine the acoustic signals of other syngnathids with a reduced or absent POC1 plate (such as *Acentronura tentaculata* and *Syngnathoides biaculeatus* in the present study). Considering that the sound producing mechanism is of a single stridulation (Chapter 4), it can be hypothesized that both (reduced and absent POC1) mechanisms would result in comparable signal shapes, similar to that of seahorses, with the acoustic parameters determined by the surface roughness of the stridulating plates (SOC-POC2).

6.2.2 Distress growls

The distress growls of seahorses were also elucidated in this dissertation. However, growls in pipefishes could not be recorded due to the fragile nature of the pipefishes. The production of growls in seahorses is accompanied by the vibration of the body. Similarly, distress vibrations were observed during handling of all pipefishes in this study. As discussed in Chapter 3, the low frequency sinusoidal component observed in combination with the feeding click is attributed to the purr as a result of stochastic resonance from the cranium during head flexion to feed. Despite no growls being recorded for pipefishes, similar purr components were detected. Thus, it can be assumed that pipefishes produce similar distress growls as seahorses.

The recorded growls and purrs in this study are consistent at the range of 150 – 200 Hz throughout the sampled species. The frequency range also matches with the reported growl frequency range of *Hippocampus reidi* (Oliveira *et al*., 2014). While anecdotal accounts of seahorse quivering, due to duress and during courtship, have been reported (Dufossé, 1874; Vincent, 1994; Masonjones and Lewis, 1996; Anderson, 2009; Ripley, personal communication; Giwojna, personal communication), it is only recently that Oliveira *et al*. (2014) documented the distress growls. Oliveira *et al*. (2014) suggested that some species of seahorses were able to produce this sound type during courtship and in stressful situations. However, courtship behaviour in syngnathids was not covered in this dissertation and based on the lack of reports on the matter, it could not be confirmed if the growl type signals are similar with the reported quivers observed during courtship. Additionally, Anderson and Mann (2011) investigated the hearing sensitivities of *Hippocampus erectus* and demonstrated that seahorses are typically characterised as hearing generalist fishes, being most sensitive to frequencies below 600 Hz and having the highest sensitivity at at 200 Hz. Interestingly, this region coincides with the frequency region of the growl and purr signals ranging between 150 – 200 Hz, leading to the question: what is the significance of the distress growl in the ecology of syngnathids?

Distress sounds have been frequently discussed in various animals (Fish and Mowbray, 1970; Walkowiak, 2007; Knight and Ladich, 2014), but it remains largely under reported in fishes (Oliveira *et al.*, 2014). Yet, we are still unsure of the functional role of these growls. It can be assumed that the functional significance of the growls is to warn and protect themselves against predators or when they are alerting conspecifics (Ladich, 1997; Bosher *et al.,* 2006). The functional significance as an alarm call has since been ruled out by Oliveira *et al*. (2014) due to the low-level nature of the growls which may only be useful within a very close proximity. Alternatively, the growls can be assumed as a last attempt to startle and indicate difficulties from ingestion to the predator (Ladich, 1997; Kaatz, 1999; Knight and Ladich, 2014; Oliveira *et al*., 2014). The scutes and spines over the body of all syngnathid fishes make these fishes hard to be ingested (Harris *et al*. 2008; Kloppmann *et al*., 2007; Kleiber *et al*., 2011). Moreover, the growl vibrations would cause serious harm to the predators in their attempt to swallow these fishes. In some species such as *Hippocampus spinosissimus*, *Hippocampus barbouri*, *Dunckerocampus* spp. and *Halicampus* spp., the edges of the bony plates are lined with scutes with some elongated to spines (Dawson, 1985; Lourie *et al*., 2004).

6.3 Evolution of acoustic signals and mechanisms

The evolution of acoustic signals and its sound producing mechanisms is influenced by selective forces shaped by adaptation and constraints related to phylogeny and morphology (Ryan, 1986). The phylogenies reconstructed with acoustic signatures corresponds with the phylogenies based on morphological and molecular data as demonstrated in other animal groups such as anurans, birds and mammals (Cocroft and Ryan, 1995; Peters and Tonkin-Leyhausen, 1999; Laiolo and Rolando, 2003).

The sound production of syngnathids have been documented in at least fifteen species (including six syngnathid species recorded in this dissertation), belonging to five different genera (Dufossé, 1874; Fish, 1953; Colson *et al*., 1998; Ripley and Foran, 2008; Anderson, 2009; Scoulding, 2011; Chakraborty *et al.,* 2014; Haris *et al*., 2014; Oliveira *et al*., 2014). Thus, the discovery and documentation of species-specific acoustic signatures and the different types of sound producing mechanisms for feeding click in aSyngnathidae provide a great opportunity to examine the evolution of acoustic communication within the fish family.

A hypothesis that can be advanced from this study is that the three-bone structure (SOC – POC1 – POC2 posteriorly) of the feeding-click producing system in the Syngnathidae is the ancestral feature, whereby, modifications of it, as observed in some pipefishes and seahorses, have been evolutionary or adaptively derived (Figure 6.2). A basis for this hypothesis could be the similar bone feature(s) as found in their primitive relatives. Previous reports on the cranial structures of the family Gasterosteidae (sticklebacks) and Pegasidae (sea moths), two families that are considered to be distant relatives of the Syngnathidae (Pietsch, 1978; Wilson and Orr, 2011; Miller *et al.* 2014), revealed that they share a similar shape of the SOC with several syngnathid species in this study (*Doryichthys deokhatoides*, *Doryichthys boaja* and *Dunckerocampus dactyliophorus*), i.e. they have a caudal extension of the SOC plate (Figure 6.4) (Pietsch, 1978; Orr, 1995; Wilson and Orr, 2011; Miller *et al*., 2014). Despite no previous documentation of the postcranial bones of sticklebacks and pegasids, the similar shape of the SOC plate serves as evidence that this structure could be of ancestral origin (Figure 6.3 B).

In the face of identifying the likelihood of common ancestral shape of the supraoccipital plate, the other morphological arrangements described in this study seem to suggest adaptive modifications (Figure 6.3). Hereby, we have inserted the reported sound producing mechanisms against the phylogenetic tree (based on concatenated 12S rDNA, 16S rDNA and Cyt*b* gene sequences) of syngnathid fishes by Wilson and Rouse (2010), and have revealed similarities in the overall grouping of syngnathids (Figure 6.5). Despite the examination of the sound producing mechanisms of only fifteen available species out of the more than 300 species of syngnathids described, the preliminary results suggest that the three-bone model in sound production is likely the ancestral form and that all the other forms are evolutionarily derived from it.

The alligator pipefish, *Syngnathoides biaculeatus*, belongs to a monotypic genus in the family Synganthidae (Dawson, 1985) and presently is the only species within the subfamily Syngnathoidinae (Wilson and Rouse, 2010). In this study, *Syngnathoides biaculeatus* is the only pipefish described so far without a POC1 plate (Chapter 4; Figure 6.3C). Could it be that the 2-bone mechanism in *S. biaculeatus* is a derived form of the three-bone model? If so, this is one form of the evolutionary or adaptive change that occurred in syngnathids (Figure 6.6). However, this argument of evolutionary change of the sound producing mechanism in *S. biaculeatus* could be more strongly supported by the presence of “intermediate forms” (e.g., having a reduced POC1) in the syngnathids.

Although the phylogenetic tree of Wilson and Rouse (2010) does not include the pygmy pipehorse, *Acentronura tentaculata*, this species also comes under the subfamily Solegnathinae (pipehorses). Examination of its sound producing mechanism shows that the POC1 plate is reduced to a small slender piece which is likely vestigial and dysfunctional in sound production (Figure 6.3D). Thus, *A. tentaculata* with a reduced POC1 provides good evidence that the 2-bone condition or mechanism as found in *S.*

biaculeatus is derived from the hypothesized, ancestral three-bone form through the disappearance of POC1.

Recent microtomographic and x-ray images by Stiller and her co-workers (2015) on three species of seadragons (*Phyllopteryx dewysea*, *Phyllopteryx taeniolatus* and *Phycodurus eques*), which belong to the subfamily Phyllopteryginae, revealed the possible possession of a different sound producing mechanism. Despite the absence of published records on the acoustic signals of these fish and their cranial bones, it can be speculated that seadragons also produce feeding click signals common throughout the family. However, further study is required to confirm if the Pylloptergina species also bears a degenerated POC1.

Among all seahorses (Hippocampinae), the observed acoustic signatures were consistently similar (Chapter 3), consistent with their joint phylogenetic grouping under the Sygnathinae based on molecular markers (Wilson et al., 2001; Casey et al., 2004; Wilson and Orr, 2011; Tsuboi, 2015). The sound producing mechanism for all seahorses retains the three-bone model but in a modified form. Here, the POC1 has evolved to form the coronet (found exclusively in seahorses). In fact the POC1, instead of becoming smaller or disappeared as in the pipefishes, has become larger extending into the vertical plane as the unique coronet. In seahorses, POC2 is retained but its role in sound producing mechanism is unknown (Figure 6.3 E; 6.5). However, the acoustic signals and energy shapes produced in seahorses due to the SOC-COR (POC1) system do not seem to suggest kinesis and contact between the coronet and POC2. Nevertheless, it seems possible that POC2 functions as a supporting buttress against the backward movement of the bulky coronet during stridulation.

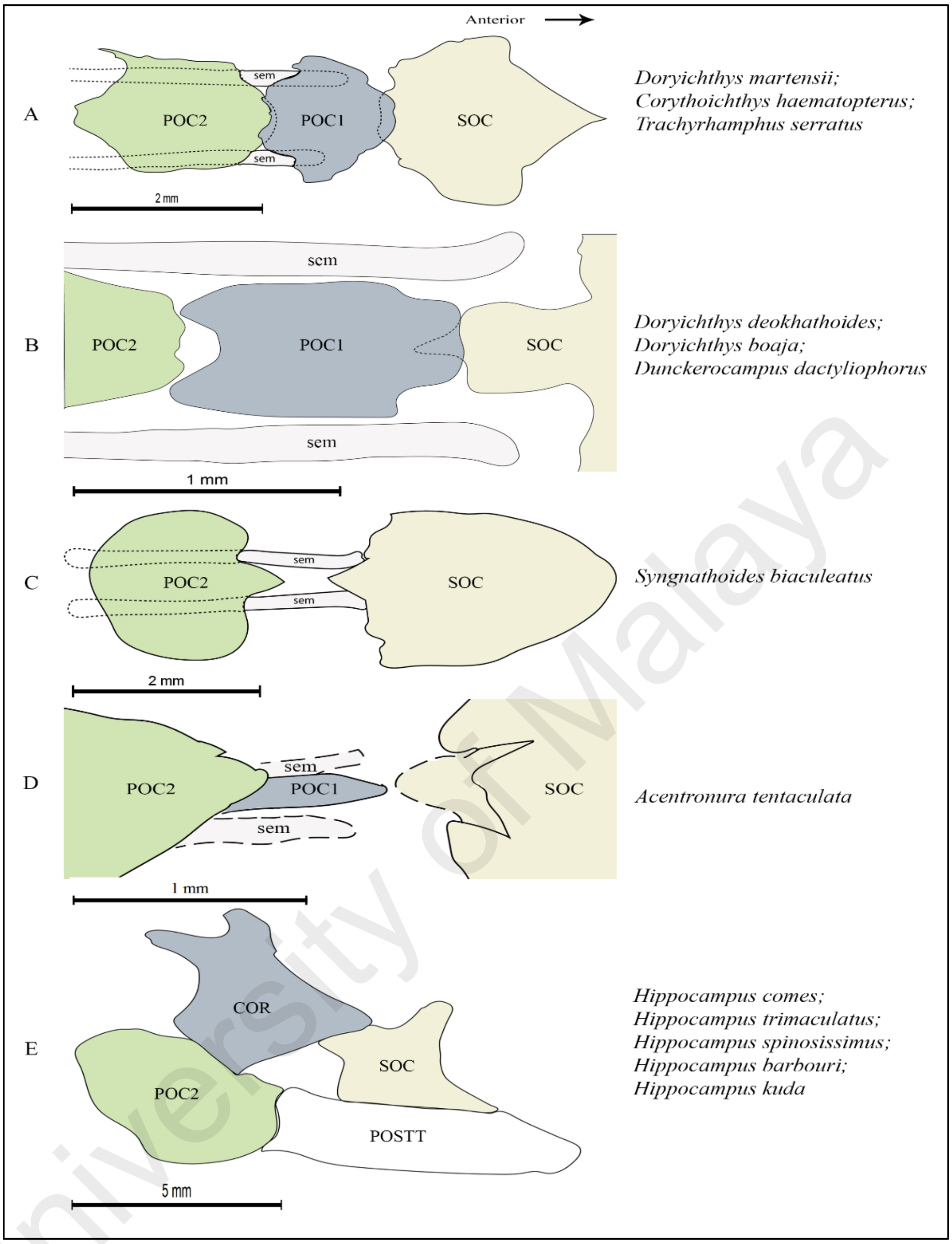

Figure 6.3: The morphological arrangement of different groups of sound producing mechanisms, A) 1st postcranial plate over sesamoid bones in epaxial tendon, posterior view, B) 1st postcranial plate between sesamoid bones in epaxial tendon, posterior view, C) 1st postcranial plate absent, posterior view, D) 1st postcranial plate reduced, posterior view, and E) COR in between the SOC and POC2, lateral view (COR, coronet; POC1, 1st postcranial; POC2, 2nd postoccipital; POSTT, posttemporal; SEM, sesamoid bones in epaxial tendon; SOC, supraoccipital).

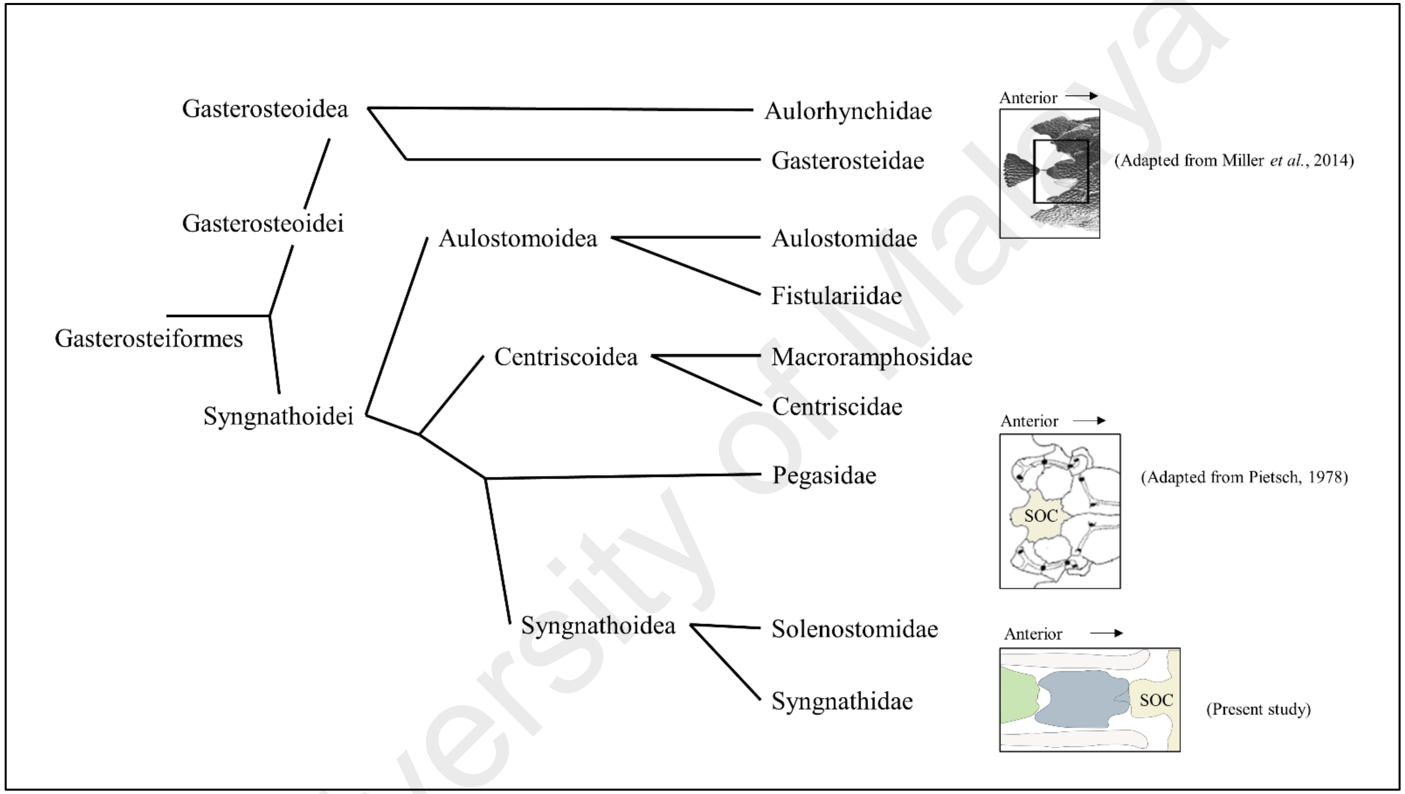

Figure 6.4: Phylogenetic relationship and superfamily classification of the Gasterosteiformes based on morphological analysis (modified from Orr, 1995) with illustrations of the supraoccipial plate of sticklebacks, Gasterosteidae (Miller *et al*, 2014), sea moths, Pegasidae (Pietsch, 1978) and syngnathids.

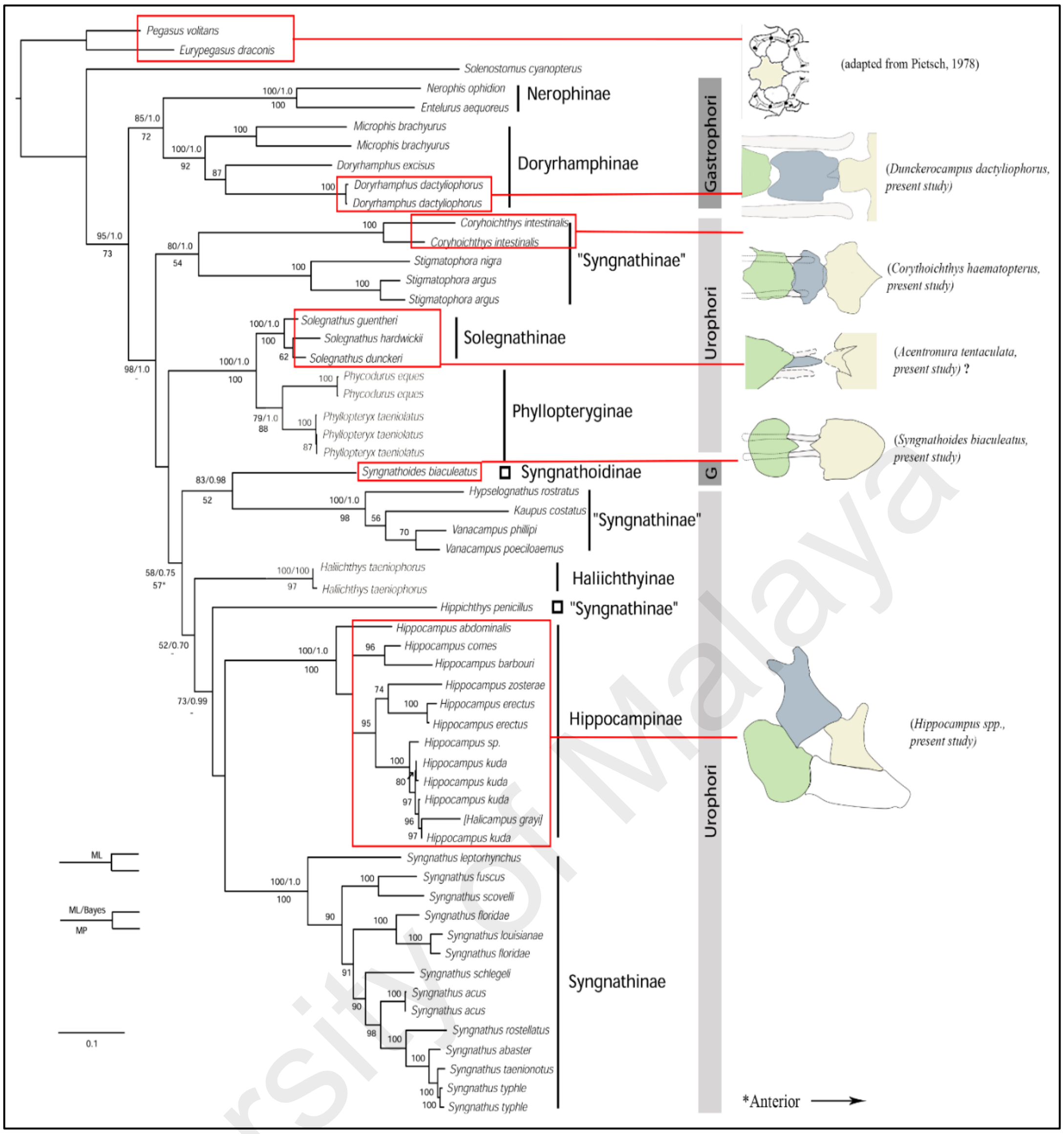

Figure 6.5: Representative sound producing mechanisms of six known subfamilies compared to other subfamilies in a maximum likelihood phylogenetic tree of syngnathids based on three mitochondrial genes (12S rDNA, 16S rDNA and Cyt*b*) (adapted from Wilson and Rouse, 2010).

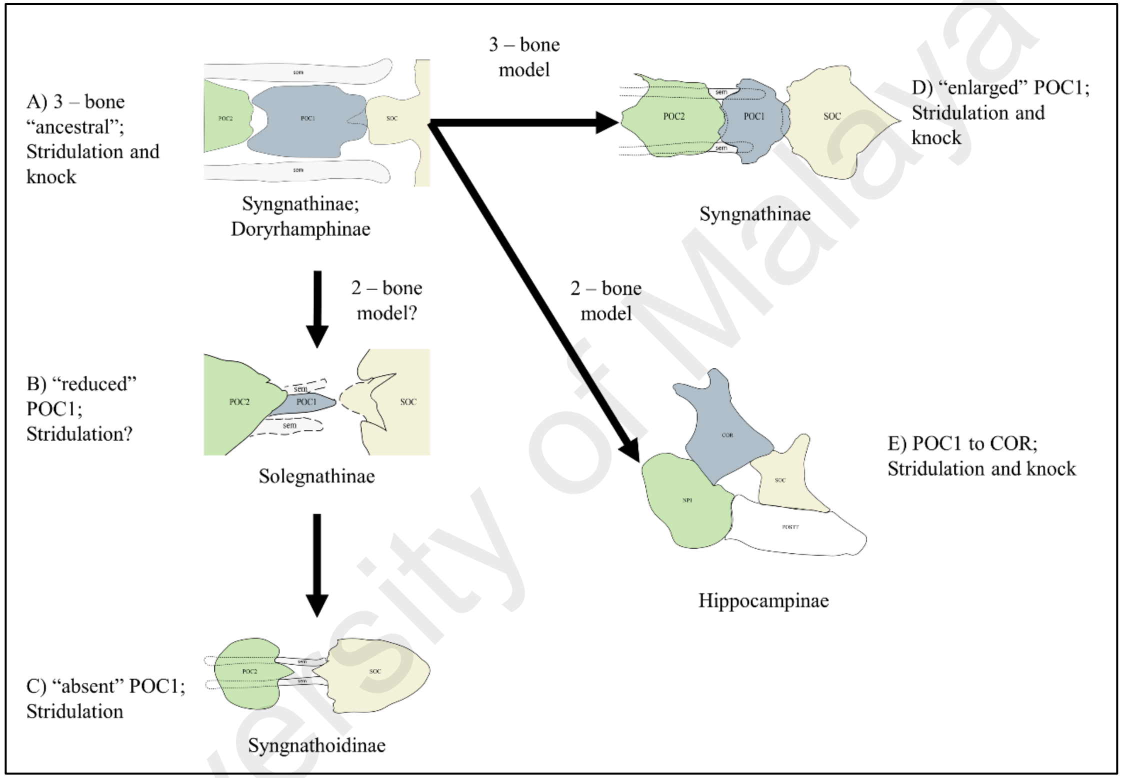

Figure 6.6: Derived sound producing mechanisms in Syngnathidae from the hypothesised ancestral three-bone model. A) "Ancestral" three-bone model with SOC, large POC1 (*Doryichthys martensii*); B) Derived form: 2 functional bones, POC1 reduced or vestigial (*Acentronura tentaculata*); C) Derived form: 2-functional bones, loss of POC1 (*Syngnathoides biaculeatus*); D) Derived form (3 functional bones), enlarged POC1, loss of caudal tail of SOC (*Corythoichthys haematopterus*); E) Derived form: 2 functional bones, POC2 likely dysfunctional, POC1 vertically enlarged as the COR. (COR, coronet; POC1, 1st postcranial; POC2, 2nd postoccipital; SEM, sesamoid bones in epaxial tendon; SOC, supraoccipital).

6.3.1 Application in syngnathid taxonomy

The results from the acoustic signatures recorded from syngnathid fishes in this dissertation could contribute to species differentiation of groups of closely related species; such as the feeding click signatures of the three species of seahorses (*H. comes*, *H. barbouri* and *H. kuda*) in Chapter 3 (Figure 6.3 E). Seahorse signatures are found to be qualitatively similar with a generic pattern due to similarities of the sound producing mechanisms, whereas, pipefishes possess multiple mechanisms leading to species-specific characteristics as a result of the variation in cranial structures. Based on the published records and the morphologies of the sound producing mechanisms of their closest relatives, it is argued that *Hypselognathus*, *Kaupus*, *Vanacampus*, *Stigmatophora*, *Corythoichthys* and *Hippichthys* described by Wilson and Orr (2011) as belonging to the Syngnathinae is erroneous. This is also supported by the molecular evidence which places the subfamily as a paraphyletic group (Fig. 6.5). Inspection of the sound producing mechanism of these genera may help to resolve the taxonomic uncertainties. For instance, it would be interesting to check out the bone mechanism of *Vanacampus* or *Hypselognathus* to see if they bear resemblance to that of *Syngnathoides* (Fig 6.5) as the closest relative.

The genus *Doryrhamphus* and *Syngnathus* are represented in fossil records dating back to the Oligocene period (estimated 23-30 million years ago) (Kotlarczyk *et al.*, 2006; Teske and Beheregaray, 2009; Wilson and Orr, 2011) and are the best possible representatives of the ancestral sound producing mechanism. While the earliest fossils of *Doryrhamphus squalidus* (Danil'chenko, 1967), *D. inolumis* (Danil'chenko, 1967), *Syngnathus incertus* (Danil'chenko, 1967) and *S. incompletes* (Korlarczyk *et al.*, 2006) indicates that the Syngnathidae family is at least 50 million years old, Wilson and Orr (2011) suggest that the family probably predates this period. The oldest known seahorse fossil dates back to the middle of the Miocene period (5-23 mya) and the genus

Hippocampus is at least 13 million years old (Žalohar *et al*., 2009). This means that the lineage of pipefishes, especially *Syngnathus* and *Doryrhamphus* predates that of the genus *Hippocampus*. Hence, the "ancestral" three-bone model (Figure 6.6) is a plausible hypothesis not contradicted by paleontological antecedence.

The present results suggest that there is congruence between the types of acoustic signatures and their generating mechanisms with the current molecular and morphological classifications (Dawson, 1985; Mobley *et al*., 2011; Wilson and Orr, 2011). For instance, Malavasi *et al*. (2008) concluded that both genetic and sound signal features portrayed similarities in the overall taxonomic grouping of sand gobies. Temporal features of sound such as frequency, duration and patterns of inter-specific variation have also been identified to contribute to the taxonomic grouping of sand gobies (Malavasi *et al*., 2008). Similarly, such acoustic parameters could be applied to syngnathid fishes to further resolve their taxonomic positions and to elucidate the evolution of signal variations.

The present study supports the hypothesis of the sound producing mechanism associated with click in syngnathids has evolved from the ancestral form, the three-cranial bone model. Regrettably, due to the limited information on acoustics and sound generating mechanisms of syngnathids, a more comprehensive and conclusive phylogenetic relationship could not be supported at this point. As the structure of the three bones (SOC, POC1, POC2) play a crucial role in the acoustic parameters, a phylogenetic tree of the syngnathids using osteological characters of these three bones should be constructed with the topology of the tree derived from molecular evidence. Thus, further investigations on the sound producing mechanisms in more syngnathids are warranted.

6.4 Time-frequency distribution analysis for bioacoustics

The acoustic recordings of syngnathid fishes in this study were analysed using the Morlet wavelet analysis approach. The Morlet wavelet analysis is commonly used in science and engineering, including the field of ecology and population dynamics (Platt and Denman, 1975; Van Wassenbergh *et al*, 2008). The wavelet transform approach executes a local time-scale decomposition of the signal to overcome the problem of non-stationarity in time series, i.e. the estimation of its spectral characteristics as a function of time (Lau and Weng, 1995; Torrence and Campo, 1998). The wavelet decomposes complex information to its basic forms at different positions and scales and subsequently reconstructs it with higher precision (Sifuzzaman *et al*., 2009). This approach also permits the tracking of different scales related to the periodic component of the signal change over time. As recognised by Cazelles *et al*. (2008), the resourcefulness of the wavelet approach lies in its decomposition properties, essentially on its time-scale localisation. Cazelles *et al*. (2008) also displayed that it is possible to study irregular, non-stationary and noisy time series along with the ability to analyse weak and transient interactions between such series. Therefore, the time-frequency approach is a powerful tool that allow the analysis of temporal and spectral distributed information content of the signal simultaneously.

In this dissertation, the wavelet transform approach was applied to analyse the bioacoustical recording of syngnathid fishes. Bioacoustic signals of marine fishes are usually evaluated using the Fourier Transform method. The wavelet transform approach has the advantage over the Fourier Transform whereby the wavelet transform not only allows the characteristic frequencies of periodicities to be determined, but also allows the localization on the time axis to be detected while the standard Fourier Transform is only localised in frequency domain (Ippolitov *et al*., 2002; Sifuzzaman *et al*., 2009). This approach also provides a better interpretation of bioacoustic recordings by allowing an intrinsic look into a signal without discarding noise. The wavelet transform is a convenient tool for compressing or de-noising a signal without appreciable degradation (Sifuzzaman *et al*., 2009; Adamczak *et al.*, 2010). The output of this approach provides an optimal simultaneous time-frequency resolution using superior analytical capabilities to study irregular signals. This approach is suitable to study signals with singularities and noises as compared to a short time Fourier Transform function with fixed frequency resolution using a fixed window (e.g. sonogram). Thus, the scalogram provides better resolution for the signals recorded.

Through the results of this approach, we were able to distinguish the different sound types and components within an acoustic signal. If a signal is oscillatory in nature, it will have a temporal distribution that is horizontally distributed and a localised frequency with an extended temporal spread of the spectrum (e.g. in *Syngnathoides biaculeatus*). Conversely, compression and pulse like signals will be localised in time with an energy spread over a broad frequency (e.g.in *Doryichthyis* sp and *Hippocampus* sp.). Hence, the scalogram energy output was able to display the species-specific acoustic signatures of the fishes. On top of spectrally differentiating the species-specific signatures, this approach also allows one to distinguish the dynamics of the events, whereby, in this study, the acoustic events were corroborated with the sound producing mechanisms of the syngnathid fishes.

6.5 Limitations of study

The limitations of this study are as below: -

1) This project could have benefitted more from a larger sample size of equal sex ratio used for the audio recordings. Unfortunately, the numbers of syngnathid fishes obtained from the local traders could not be controlled due to their limited availability throughout the duration of the project. As a result, the availability of syngnathids for the

conduct of experiments was irregular. Specific species could not be targeted, and mixed species samples of a few individuals were the norm. The problem of small sample size was further compounded by occasional fish mortality due to stress during transportation.

2) By using an engineering approach, the location of the seahorse growls has been identified and pinpointed to the cheek region of the seahorse. Despite narrowing down to the closest location of the growl generating mechanism (at the cheek region), the actual mechanism and its sound generating activity still cannot be determined in the study.

3) Pipefishes were also observed to produce growls when in distress but not on a consistent basis. The growls of pipefishes could not be recorded due to their fragile nature. Several attempts were made using the available live specimens. Unfortunately, the attempts have led to high mortality of the pipefishes. Therefore, no further attempts were made to induce stress for sound recording.

4) Microtomographic digital images of other syngnathid species could have been taken to provide a better understanding and comparison of the sound producing mechanisms. The Skycan microtomographic services unit provided by Malaysia Nuclear Agency was limited by the size of the scan samples. Syngnathid head samples larger than the accepted size of the microtomographic unit could not be scanned and reconstructed.

5) The documentation of syngnathid diversity in Malaysian waters was limited by the disapproval of local sampling permits in marine protected areas by Marine Parks Malaysia. To cope with the permit rejection, we focused on local landing ports and images collected by citizens, mainly scuba divers for syngnathids which were captured within Malaysian waters.

6.6 Future research recommendations

The present study has documented the diversity of sound characteristics and sound producing mechanisms in the syngnathid fishes, thereby opening up a vast scope for future investigation. The mapping of the acoustic characteristics and sound producing mechanisms of other syngnathid species would provide us with a greater understanding of its diversification.

Further examination of the three-bone mechanism (SOC-POC1-POC2) of the cranial region of other syngnathid fishes would help unravel the taxonomic and phylogenetic significance of the sound producing mechanism in the family. Examination of members of the following subfamilies are warranted: Nerophinae, Phyllopteryginae, Halichthyinae and the dubious “Syngnathinae” (Fig. 6.5). In fact, examination of other species of those subfamilies has already been done in the present study is advocated. The differences in sound producing bones appear to be an important factor that could have been evolutionary or adaptively derived. Further investigations on the evolution of sound producing mechanisms are required to justify this theory.

In the present study, the exact location of the growl emission was identified using an accelerometer, which found that the sound originated from the cheek of the fish. Unfortunately, despite identifying the location of the growl, we could only hypothesise that the growls could possibly be produced from the vibrations of the pectoral-pelvic girdle. Therefore, future investigation on the growl emitting mechanism should be capable of accurately describing it. It is also important to catalogue the species-specific signatures for species identification purposes.

Investigations on the response of these fishes to acoustic playbacks using the pre-recorded sounds of conspecifics and allospecifics present an interesting area of study. Any behavioural response to the acoustical playbacks could aid our understanding of the significance of fish sounds in communication in this charismatic group of fish.

6.7 Conclusion

The time frequency analysis of post-proceessed syngnathid bioacoustics signals successfully improves the resolution output and significantly increases the understanding of syngnathid sound production. The improved output resolution makes it possible to discover for the first time the multiple components within the syngnathid feeding click. The multiple components consist of high and low frequency components, and occasionally, another low-frequency component called the purr. The acoustic parameters (frequency and time) in syngnathids are influenced by the different sound producing mechansims which appear to be species-specific in six species examined. The feeding click in syngnathids results from cranial bone kinesis that generates stridulating and knocking sounds. Three bones or cranial plates participated in sound generation, namely, the supra-occipital (SOC), the first post-occipital plate (POC1) and the second postoccipital plate (POC2), posteriorly. It is hypothesized that the three-bone mechanism or SOC-POC1-POC2 model is the ancestral sound-generating mechanism of syngnathids, and that all extant syngnathids either maintained this model or have evolutionary or adaptively derived other models from it. Such varied morphologies and acoustic differences may benefit species identification.

The distress growl has a single low frequency component generated in the cheek region of the fish; this study was unable to determine the generating mechanism, but it is speculated to be the pectoral girdle. The growl's acoustic parameters (frequency and duration) also appear to be species-specific.

The Malaysian syngnathids inhabit many aquatic habitats including coral reefs, mangroves, seagrass meadows, seaweeds, sponges and octocorals, estuaries, rivers and streams, sandy bottoms and muddy bottoms. Despite the highly varied environments inhabited by these fish, there is no evidence that their acoustic signals are related to their habitat.

The findings from this dissertation offer vast scope for future investigation. The results address important questions in the signal generation and signals of syngnathid fishes. Future studies should focus on examining the sound producing mechanisms and their associated acoustic signals for potential taxonomic significance. Upcoming investigations should be capable of accurately documenting the sound producing mechanisms of the distress growls in seahorse and other fragile syngnathids. This study has enhanced our knowledge of bioacoustics of syngnathid fishes and may influence the way we study their behavioural and communicative ecology in the future.

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