5.0 DISCUSSION

5.1 SPATIAL DISTRIBUTION OF PHYTOPLANKTON

The number of phytoplankton species recorded from ST1 was lower than other stations. Phytoplankton community at ST1 consisted of estuarine and freshwater species. However, phytoplankton communities at ST2 and ST3 consisted of estuarine, freshwater and marine species.

Cell accumulative abundance which is the mean of cells abundance in 12 months was significantly higher at ST2 and ST3 as compared to ST1. According to Rajkumar *et. al*. (2009) marine phytoplankton species are transported to mangrove by tidal activities. It was noted that phytoplankton species such as *Thalassionema nitzschioides*, *Bacteriastrum comosum* and *Chaetoceros* spp. that were found at ST2 and ST3 were absent from ST1. According to Muylaert, *et. al*. (2009), these three species are essentially marine species. Perumal *et al*. (2009) and Senthilkumar *et al*. (2002) in their studies suggested phytoplankton species composition was normally high at the rivers mouth due to high variation in hydrographical conditions. Earlier study by Normawaty (1998), reported that phytoplankton cells accumulate at river mouth because of the area is rich with nutrient.

Most of phytoplankton species are common to 2 among the 3 study stations. *Melosira nummuloides*, however, was only found at ST3. This centric chain-forming species of order Melosirales is commonly observed and sampled at marine area (Thomson, *et. al*., 1980) and in latter study in both marine and estuarine areas (Muylaert, *et. al*., 2009). This was the first record of the occurrence of *M. nummuloides* at Carey Island. Previously, Salleh and Tajuddin (2006) reported *M. moniliformis* as the only species of genus *Melosira* to inhabit Carey Island aquatic ecosystem. Relatively high cells abundance (7.32 x 10^5 cells/L) of the species was recorded in July 2009, in which the highest water temperature and the lowest dissolved oxygen at ST3 were detected. The ability of *M. nummuloides* to tolerate increased temperature and to utilize decreased dissolved oxygen (Rendall *et. al*., 1986) due to competition with other phytoplankton species enables them to survive the condition.

Peridinium cinctum of division Pyrrophyta was the species with the lowest RD with the value of only 0.03%. It was found inhabiting both ST2 and ST3 in relatively low density. Surprisingly, although the species is known as freshwater species (Wynne, 1981), there was zero occurrence of the species at ST1 which was located upstream Langat River. Demands on sufficient amount of phosphorus concentration that was relatively high at both ST2 and ST3 must be one of crucial factors of this phenomenal. Grigrorszky *et. al*. (2006) reported that phosphorus is vital for *P. cinctum* growth and also is one of important factors controlling its encystment process in warmer condition. Earlier study by Wynne (1981) suggested phosphorus is important for *P. cinctum* to synthesize phosphatase to carry out metabolic processes for its growth and reproduction.

Skeletonema costatum was the species with the highest RD, 49.23% and was dominant at all stations with the highest abundance at ST3, with mean density of 4.93 x 10^5 cells/L. Most of researches on phytoplankton and microalgae done in mangrove and estuary proved that *S. costatum* is the common species to inhabit the ecosystem (Chen, *et. al*., 2010; Patil, *et. al*., 2008; Perumal, *et. al*., 2006; Lassen, *et. al*., 2004). The species is always responsible for red tide occurrence at ocean and estuarine (Liu, *et. al*., 2005). Onsets of *S. costatum* red tide often triggered by decreasing salinity, increased temperature and climate disturbance such as rainfall (Weng, *et. al*., 2011; Liu, *et. al*., 2005; Zhu, *et. al*., 2005), since it normally can adapt well in such condition compared to other phytoplankton species (Lomas & Gelibert, 2000).

5.2 TEMPORAL DISTRIBUTION OF PHYTOPLANKTON

Major findings from studying the temporal distribution of phytoplankton are that phytoplankton abundance (cells/ml) and species richness showed significant difference between tides and between months. Both abundance and species richness were strongly affected and influenced by factors such as bloom, weather, nutrients availability, hydrographic condition and also grazing activity.

5.2.1 Phytoplankton Distribution in High Tide and Low Tide

Study showed high density of phytoplankton in high tide compared to low tide, in which the abundance could double or triple the abundance sampled in low tide. For instance, at ST3 during the first sampling month, high tide's cells abundance was recorded 2.67×10^6 cells/L while in contrast only 4.35×10^5 cells/L occurred in low tide. Brunet and Lizon (2003) suggested phytoplankton able to increase their growth rate during high tide, since they receive ample light intensity to carry out photosynthetic activities. Study by Chaneva *at. al*. (2007) however, proved a very high light intensity will destruct the photosynthetic pigment thus decreased photosynthetic activities. However, since the study was done in mangrove, light is not the main factor to affect phytoplankton abundance (Owen, 2004). Thus, light intensity was not recorded in this study.

At study site, salinity might be one of important physical factors that reflected the different between abundance and species richness in both tides. Sridhar *et. al*. (2006) suggested that salinity can acts as limiting factor that able to influence phytoplankton distribution. Generally, changes in salinity in mangrove waters are caused by tidal variations (Perumal, *et. al*., 2009). Though Pearson correlation showed non-significant correlation between phytoplankton and the parameter, it displayed moderate non-linear relation which means, salinity intercorrelated with other physical parameter in order to play its role. A study by Pilkaityte *et. al*. (2004) proved salinity together with temperature will enhance phytoplankton growth at estuary area. Relatively high salinity level in ambient temperature level (ranged between 28°C - 33°C) during high tide enhanced the growth of phytoplankton diversity at study site.

In order to carry out their metabolic processes for growth and reproducing, phytoplankton needs sufficient concentration of nutrients. However, nutrients concentration in both tides fluctuated throughout a year of sampling. Therefore, it was quite impossible to conclude which nutrient responsible for the difference in phytoplankton abundance and diversity between both tides.

However, based on Pearson Correlation test, some predictions were made. r values displayed nitrate correlated strongly with cells abundance in high tide (r=0.852) compared to low tide (r=0.637). The r strength indicates nitrate concentration played vital role in both tides. However, the species occurred in low tide did not respond well to this nutrient. According to Anderson *et. al.* (2001), there were 2 sources of nitrate at estuarine, which are from tidal mix and rainfall runoff or both. It shall be concluded that source of nitrate at study site was mainly from rainfall runoff and not the tidal mix, since the highest nitrate concentration did not only recorded during high tide but also in low tide.

Silicate concentration has always been related to phytoplankton of division Bacillariophyta. Diatom was abundantly being sampled during high tide compared to low tide. Studies have proved that this division correlates positively with silicate concentration (Olli, *et. al*., 2008; Lassiter, *et. al*., 2006). However, at study site, though cells abundance of Bacillariophyta was relatively high during high tide, the silicate concentration was recorded lower (ranged between 0.50 mg/L - 8.75 mg/L) compared to its concentration in low tide (ranged between 6.00 mg/L - 27.45 mg/L).

Decrement of water volume during low tide was responsible to increase the silicate concentration during low tide compared to high tide. Besides, since the Bacillariophyta and other phytoplankton species were relatively low during low tide, the silicate uptake might be lower thus silicate remained in water column. To further verify this, Olli *et. al*. (2008) proved dissolved silica experienced gradual depletion when there is occasion of monospecific bloom. Hence, put pressure on other phytoplankton species especially the diatoms. However, normally, dissolved silica concentration shows increment after the bloom ends. The finding by Olli *et. al*. (2008) reflects that a relatively high abundance of phytoplankton cells moreover the diatoms in high tide at study site, decreased the silicate concentration.

Pearson correlation (r) value between silicate and phytoplankton abundance during high tide (r=0.849, p<0.01) displayed a positively strong significant correlation hence supported silicate concentration is crucial for phytoplankton growth during high tide which dominantly comprised of division Bacillariophyta. On the other hand, the r value during low tide displayed moderate positive correlation (r= 0.585, p<0.05). Still, the value indicates silicate has influenced the growth of phytoplankton during low tide though in low impact.

Despite of high species number and phytoplankton cells' abundance during high tide, there was species of phytoplankton that only found to occur in low tide whereas absent from high tide. *Oscillatoria tenuis* was the species sampled during low tide in relatively high density.

O. tenuis of Cyanobacteria occurrence in low tide predicted to have close relation to high sulfate concentration during low tide. The observed result at study site is further supported by research done by Slooten *et. al*. (1989), proved genus *Oscillatoria* managed to go on photosynthesis with either water acts as electron donor in aerobic condition or sulfide in anaerobic condition. However, certain *Oscillatoria* species also use sulfide as electron donor though in aerobic condition (Fike *et. al*., 2009). To further verify this, Shabana *et. al*. (2000) reported 5 Cyanobacteria strains which includes genus *Oscillatoria* showed response to sulfide in both aerobic and anaerobic conditions. However, the response depends on the species, time exposure and sulfide concentration. *Oscillatoria tenuis* at study site may use both water and sulfide as electron donor to carry out photosynthesis during low tide.

Besides, as long chained filamentous Cyanobacteria, it makes them susceptible to sink at the bottom of water body. Thus, the species can be sampled only during low tide at study site. El Herry *et. al*. (2008), suggested that *O. tenuis* was particularly sampled at bottom water (more than 9m depth). Earlier study by Foy & Gibson (1982), found the species needs to inhabit lower or bottom water since they require low light intensity to grow. Higher temperature during low tide (up to 36 ºC) also makes it favorable for the species to grow and be sampled during low tide. In Egypt and Algeria, *O. tenuis* bloom is commonly detected during the warmest month which normally in summer (Nasri *et. al*., 2004; Mohamed *et. al*., 2003). However, there is no study that specifically discussed on the reason of this species affinity towards higher temperature compared to lower temperature.

5.2.2 Phytoplankton Distribution in Different Months

In terms of species composition, the tropical climate of study site was reflected by the occurrence of many tropical species in warm condition. In addition, species composition also represented and evidenced most phytoplankton species were the common species appeared and sampled at mangrove, marine and freshwater ecosystems. Location of study site that was situated in mangrove in an estuarine enables diverse species to be sampled from the area.

Temporal distribution of phytoplankton at study site was strongly influenced by climate. Latter studies by Patil *et. al*. (2008), Lee *et. al*. (2009), Nahar *et. al*. (2010) on both temporal and seasonal distribution of phytoplankton all around the world are some best examples to further verify the observation. Study on temporal patterns by Philips *et. al*. (2010) discussed on the effect of major issues of climate change which was the global warming on phytoplankton composition. They suggested that temporal changes (increased temperature) in climate within a year able to deviate the composition of phytoplankton. Warmer condition enhances the growth and bloom of *Pyrodinium bahamense* which totally alters the phytoplankton composition of study area.

Climatic or weather change at study site affected the phytoplankton distribution and occurrence by means of rainfall that normally will directly or indirectly influence the phytoplankton (Philips *et. al*., 2010). As previously discussed, rainfall is responsible to cause accumulation of nutrient in mangrove ecosystem water. Study site that was located in the vicinity of oil palm plantation, may received high concentration of nutrients originated from fertilizer and the use of herbicides to control weeds, such as nitrate and phosphorus.

Temporally, dynamic correlation between nitrate and cells abundance was detected at study site. As discussed previously, correlation coefficient displayed strong relation between nitrate and cells abundance with $p<0.01$ (during high tide) and $p< 0.05$ (during low tide). On the other hand, correlation coefficient between phosphorus concentration and cells abundance showed significant relationship only during low tide with $p<0.01$ and $p<0.05$.

Data of cells abundance and chemical parameters of study site evidenced though rainfall affected the phytoplankton abundance at study site, observation proved that only light rainfall enhanced phytoplankton abundance. For instance, light rainfall in July

2009, August 2009 and March 2010 coincided with relatively high cells abundance. Whereas heavy rainfall during night before sampling day in October 2009 and heavy rainfall during sampling day in January 2010 resulted in low nitrate and phosphate concentration coincided with low cells abundance. Philips *et. al*. (2010), in their study found high or heavy rainfall able to wash away pivotal nutrients for phytoplankton out of estuary and lagoon ecosystems. Hence, there will be no sufficient nutrients to enhance and stimulate phytoplankton growth. In addition, Patil *et. al*. (2008) reported heavy rainfall helps in declining blooms of *Skeletonema* and *Fragilariopsis* at monsoon-influenced tropical Zuari estuary in India.

In early sampling months, which was carried out in both April 2009 and May 2009, recorded relatively high cells abundance with mean density of 2.36×10^6 cells/L and 2.36×10^6 cells/L respectively. The hot and dry weather during sampling days may responsible to stimulate the growth of phytoplankton. The relatively high temperature was reflected by the high cells abundance with both April 2009 and May 2009 mean reading recorded as 31.17 ºC and 31.16 ºC respectively. More than 80% of total densities of phytoplankton in both months were comprised of *Skeletonema costatum*. High temperature with addition of high nitrate concentration believed was responsible to enhance the growth of *S. costatum* hence increasing the total density of phytoplankton in those particular months. Liu *et. al*. (2005) and Olli *et. al*. (2008) also proved high temperature able to enhance the growth of the species when they did study on diatom and phytoplankton succession in Jiaozhou Bay, China and Baltic Sea respectively. While study done by Anderson *et. al*. (2001) in Western Long Island Sound on effect of nutrient pulses on phytoplankton blooms further verify that *S. costatum* growth is directly proportional to nitrate concentration.

Nevertheless, long water residence time with slow current in the mangrove may responsible to enhance algal bloom occurrence at study site. To further verify this, Vila and Maso (2005) suggested in open environment such as river or estuarine, elevated water residence time able to enhance the duration of certain species of phytoplankton to inhabit the ecosystems which normally will trigger bloom. Later study by Philips *et. al*. (2006) stated long water residence time is the major factors that able to increase the frequency or intensity of bloom to happen. At study site, slow current at mangrove area of Carey Island may responsible to retain nutrient that essential for the phytoplankton to go on living processes.

There were two phytoplankton genera that responsible to cause bloom at study site which were the *Skeletonema* and *Pinnularia*. Bloom occasion at study site was defined as the density of causative species reached 1.00×10^6 cells/L (Lee *et. al*., 2009). *Skeletonema costatum* was the species that caused bloom in 3 sampling months, April 2009, May 2009 and August 2009 out of 12 sampling months, with mean density of 1.65×10^6 cells/L, 1.81×10^6 cells/L and 1.46×10^6 cells/L respectively. The bloom occurrence was responsible to cluster the species composition of the 3 months into same group when tested with UPGMA Modified Morisita's Smilarity Index.

However, *S. costatum* bloom proved to only cause minor changes to the species composition of phytoplankton since despite the bloom occurrence, the species composition in April, May and August 2009 still shared more than 80% similarity with other sampling months' species composition except with July 2009. Specifically, high temperature and ambient salinity may have provided suitable condition for *S. costatum* to undergo growth processes. Observed during the occurrence of *S. costatum* bloom, water at study site turned into dark brown color. The color of the study site was darker concurrently to the density of the species. Liu *et. al*. (2005) and Lee *et. al*. (2009) also recorded same observation on the color of *S. costatum* bloom.

On the other hand, the outbreak of *Pinnularia* species was recorded in July 2009. There were 2 species that responsible for the blooms which were *Pinnularia acuminata* and *Pinnularia tabellaria* which mean density of 1.35 x 10^6 cells/L and 1.075 x 10^6 cells/L. The relatively high density of genus *Pinnularia* at study site in July 2009 reduced the number of species with average species number of only 9 species. The *E* value was 0.211 while the H' value was 0.668 during the outbreak. The outbreak was also responsible to separate the species composition in July 2009 from other months with 0% of species similarity. Bloom of *Pinnularia* may responsible to inhibit the growth of other phytoplankton species, thus, altered the species composition in July 2009. On the occurrence of the bloom, salinity was ranged between 23.8 ppt – 26.1 ppt, while temperature was recorded between 29.56 ºC – 32.34 ºC, followed by light rainfall with crystal clear water.

Order Chaetocerotales was the most diverse group of phytoplankton with 20 species occurred at study site. Surprisingly, species of order Chaetocerotales were only occurred in the first 3 months (April 2009, May 2009 and June 2009) of sampling and the final 3 months (January 2010, February 2010 and March 2010) of sampling, with reemergence in August 2009. However, the density of this order was not high to trigger and cause bloom. Contrarily to study done by Ishikawa & Furuya (2004) reported *Chaetoceros* is not only dominant species but is the common order to cause blooms in estuaries.

The occurrence of high density of shrimp larvae that was observed during sampling days in September 2009 until December 2009 was the best explanation to further verify the finding. The shrimp larvae were responsible to feed on the species of order Chaetocerotales. Borowitzka *et. al*. (1997) and Hemaiswarya *et. al*. (2011) reported that Chaetocerotales has been used in aquaculture field as the best food for mollusks, crustaceans and rotifers. Besides functioning as food for the shrimp, Hemaiswarya *et. al*. (2011) also stated that the genus is also important to indirectly increase the vitamin levels in shrimp hatchery location. However, unfortunately, there was no detailed data and information was done on the correlation between phytoplankton and grazing effect by those shrimp larvae at study site.

The occurrence of order Chaetocerotales in January, February and March 2010 resulted the months to be clustered under same group in cluster analysis. However, months of April and May 2009 were not clustered under the same group as the mentioned 3 months though there was also occurrence of order Chaetocerotales in both sampling months (Figure 4.18). Low density of *S. costatum* in the 3 final sampling months was responsible to separate these months from being clustered under same group with the species composition in April and May 2009. Heavy and light rainfall that happened in January, February and March 2010 may responsible to decrease the abundance of *S. costatum* from the sampling months. On the other hand, order Chaetocerotales that also known as stenothermal and stenohaline species by Lee *et. al*. (2009) is able to survive the high precipitation which altered not only the temperature and salinity but also nutrients of the mangrove area.