

Monitoring of Water Quality and Microalgae Species Composition of *Penaeus monodon* Ponds in Pulau Pinang, Malaysia

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Abstrak: Laporan terdahulu telah mendedahkan bahawa kelimpahan mikroalga di dalam kolam udang adalah berbeza-beza dengan perubahan faktor persekitaran seperti cahaya, suhu, pH dan status nutrien sepanjang tempoh penternakan udang. Dalam kajian ini, tempoh penternakan udang dibahagikan kepada tiga fasa (awal = minggu 0–5, pertengahan = minggu 6–10 dan akhir = minggu 11–15). Faktor fizikal dan kimia sepanjang tempoh penternakan ini dikaji dan komposisi mikroalga dipantau. Faktor fizikal didapati berubah-ubah dengan nilai julat keamatan cahaya antara 182.23–1278 $\mu\text{mol foton m}^{-2}\text{s}^{-1}$, suhu antara 29.56°C–31.59°C, oksigen terlarut antara 4.56–8.21 mg/l, pH antara 7.65–8.49 dan saliniti antara 20‰–30‰. Kepekatan amonium ($\text{NH}_4^+\text{-N}$), nitrit ($\text{NO}_2^-\text{-N}$), nitrat ($\text{NO}_3^-\text{-N}$), dan ortofosfat ($\text{PO}_4^{3-}\text{-P}$) di kolam udang pada semua fasa penternakan setiap satunya mempunyai julat bacaan antara 0.017–0.38 mg/l, 0.24–2.12 mg/l, 0.06–0.98 mg/l dan 0.16–1.93 mg/l masing-masing. Ujian statistik (ANOVA) menunjukkan bahawa tidak terdapat perbezaan yang signifikan ($p < 0.05$) terhadap kepekatan nutrien antara fasa-fasa penternakan tersebut. Walau bagaimanapun, semua kepekatan nutrien tersebut masih berada dalam julat bacaan yang selamat untuk penternakan udang. Kepekatan klorofil *a* menunjukkan julat bacaan antara 5.03 ± 2.17 hingga $32.61 \pm 0.35 \mu\text{g/l}$ sepanjang tempoh penternakan. Sebanyak 19 spesies mikroalga ditemui di kolam udang. Daripada jumlah itu, 72% adalah diatom, diikuti oleh Chlorophyta (11%) dan Cyanophyta (11%). Namun, kelimpahan spesies mikroalga adalah berbeza-beza pada setiap minggu sepanjang tempoh kajian. Pada fasa awal, ketika udang belum dimasukkan ke dalam kolam, *Anabaena* spp. dan *Oscillatoria* spp. (Cyanophyta) merupakan spesies yang dominan di dalam kolam, diikuti oleh *Chlorella* sp. dan *Dunaliella* sp. (Chlorophyta). Apabila udang dimasukkan ke dalam kolam, spesies diatom iaitu *Amphora* sp., *Navicula* sp., *Gyrosigma* sp. dan *Nitzschia* sp. didapati mula wujud di dalam kolam. Pada fasa pertengahan dan menuju fasa akhir penternakan udang, diatom didapati mendominasi kolam udang. Spesies Chlorophyta (*Chlorella* sp.) mendominasi sebanyak 2 kali, iaitu pada minggu ke 2 dan 13. Ketidakhadiran beberapa spesies mikroalga air masin di dalam kolam udang kemungkinan besar disebabkan tidak boleh bertoleransi terhadap perubahan fizikal dan kimia persekitaran kolam yang berubah secara mendadak. Dalam kajian ini, *Cylindrotheca closterium* merupakan spesies yang paling toleran di antara mikroalga yang terdapat di kolam udang kerana kemampuannya mendominasi selama 6 minggu daripada 15 minggu tempoh penternakan.

Kata kunci: Kedominan Spesies, Klorofil *a*, Diatom, Chlorophyta, Cyanophyta

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Abstract: Many reports have revealed that the abundance of microalgae in shrimp ponds vary with changes in environmental factors such as light, temperature, pH, salinity and nutrient level throughout a shrimp culture period. In this study, shrimp cultivation period was divided into three stages (initial = week 0–5, mid = week 6–10 and final = week 11–15). Physical and chemical parameters throughout the cultivation period were studied and species composition of microalgae was monitored. Physical parameters were found to fluctuate widely with light intensity ranging between 182.23–1278 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$, temperature between 29.56°C –31.59°C, dissolved oxygen (DO) between 4.56–8.21 mg/l, pH between 7.65–8.49 and salinity between 20‰–30‰. Ammonium ($\text{NH}_4^+\text{-N}$), nitrite ($\text{NO}_2^-\text{-N}$), nitrate ($\text{NO}_3^-\text{-N}$), and orthophosphate ($\text{PO}_4^{3-}\text{-P}$) concentrations in the pond at all cultivation stages ranged from 0.017 to 0.38 mg/l, 0.24 to 2.12 mg/l, 0.06 to 0.98 mg/l and 0.16 to 1.93 mg/l respectively. Statistical test (ANOVA) showed that there were no significant difference ($p < 0.05$) in nutrients concentrations among the cultivation stages. All nutrients concentrations however were still in the tolerable level and safe for shrimp culture. The chlorophyll *a* contents were found to range from 5.03±2.17 to 32.61±0.35 $\mu\text{g/l}$ throughout the cultivation period. A total of 19 microalgae species were found in the shrimp pond, with diatoms contributing up to 72% of the species followed by Chlorophyta (11%) and Cyanophyta (11%). However, weekly species abundance varied through the study period. At the initial stage, when there were no shrimps in the pond, *Anabaena* spp. and *Oscillatoria* spp. (Cyanophyta) were the dominant species, followed by *Chlorella* sp. and *Dunaliella* sp. (Chlorophyta). When shrimps were introduced into the pond, *Amphora* sp., *Navicula* sp. *Gyrosigma* sp. and *Nitzschia* sp. (diatoms) started to exist. At the middle and towards the final stage of the shrimp culture period diatoms were the dominant species. The Chlorophyta (*Chlorella* sp.) domination took place only twice, which was at week 2 and 13. The absence of some of the coastal water microalgae species in the shrimp pond was most likely due to the fact that they could not tolerate the physicochemical factors of harsh environment. In this study, *Cylindrotheca closterium* was regarded as the most tolerant species among the microalgae due to its ability to exist for 6 weeks out of the 15 weeks of cultivation.

Keywords: Species Dominance, Chlorophyll *a*, Diatom, Chlorophyta, Cyanophyta

INTRODUCTION

Microalgae are well known as an important source of supplement food, animal feed, bioactive compound, biofuel (Borowitzka 1999; Melis 2002; Shimizu 2003; Singh *et al.* 2005; Metzger & Largeau 2005), and are also significant in bioremediation applications (Kalin *et al.* 2004; Munoz & Guieysse 2006) and nitrogen fixation (Vaishampayan *et al.* 2001). Microalgae was suggested as a good candidate for producing biofuel because of their high lipid content, high photosynthetic efficiency, high biomass production and fast growth compared to other energy crops (Chisti 2007). Microalgae species can be found in any water body be it in freshwater or marine environment. Shrimp ponds and fish ponds are among the places that microalgae grow very well and in abundance. The physical and chemical parameters of the environment that house the microalgae will determine the species that can grow and survive well. Each of the microalgae species requires specific nutrients and physical conditions to enable them to grow healthily. Shrimp ponds offer unexhausted source of nutrients to the microalgae as well as CO_2 gas respired from shrimp metabolism. In shrimp

ponds, microalgae were found growing naturally and their diversity and abundance often vary, depending on several environmental factors such as light, temperature, pH, salinity and nutrient availability (Araújo & Garcia 2005; Alonso-Rodriguez & Páez-Osuna 2003). Microalgae require a wide variety of chemical elements but the most important are nitrogen and phosphorous for growth and reproduction (Cremen *et al.* 2007).

Since the production of biofuel requires large amounts of microalgae biomass, the current practice of commercial-scale cultivation of microalgae is very expensive and not cost effective. This hygienic culture method is meant for the production of microalgae biomass for food supplement, pharmaceutical, nutraceutical and food for zooplankton in hatcheries and not economical to be used as a biomass production for biofuel generation. Thus, our intention is to use shrimp ponds as natural photobioreactors for large scale microalgae cultivation. When the Standard Operating Procedure (SOP) is established, this mass cultivation method will not require high technical expertise to operate and is at low cost since there is no extra expenditure for light, water, CO₂ and nutrients. Using this approach, shrimp farmers will get an extra income from cultivating the microalgae.

Cremen *et al.* (2007) carried out a study to illustrate the qualitative and quantitative changes in phytoplankton communities in tropical commercial shrimp ponds using green water (microalgae) with different stocking densities. According to their study, chlorophycaceae (mostly *Nannochloropsis* sp.) is the dominant species during the initial culture phase (1–35 days), which coincide with high salinity (35.7 ppt). The cyanophycacean bloom occurred towards the final culture phase (84–112 or 126 days of culture) when there was low salinity (19.5 ppt) and a short diatom bloom occurred at the same time. Yusoff *et al.* (2002) reported that diatoms were dominant and cyanophytes were absent at the beginning of shrimp culture. After 34 days, the diatoms significantly decreased whereas cyanophytes increased. Cyanophytes were significantly high during final phase of the culture period compared to the initial culture period. According to Alonso-Rodriguez and Paez-Osuna (2003), in most ponds (intensive and semi-intensive), cyanophytes are the dominant species, followed by dinoflagellates. According to Boyd (1989), diatoms enhance shrimp growth better than cyanophytes and most shrimp farm managers prefer a high ratio of diatoms in a phytoplankton community because diatoms are beneficial algae that play an important role as a food source for aquatic invertebrates. However, not all microalgae species are beneficial to the shrimp pond ecosystem.

Monitoring chemical and physical parameters and the microalgae abundance in the shrimp pond will help us to understand the factors that control the presence (promote) or the absence (inhibit) of a certain microalgae species. Yusoff *et al.* (2002) stated that the occurrence of microalgae species such as diatoms in shrimp ponds can be temporary before it was replaced by cyanobacteria which dominated the ponds for a longer time because the increase in nutrient concentrations over the cultivation period benefited cyanobacteria. Sometimes there was a microalgae bloom for a short period of time. This microalgae blooming will reduce oxygen concentration and will affect shrimp growth. To avoid blooms, the algae have to be removed from the shrimp ponds.

The aim of this study is to monitor the physical and chemical parameters in a shrimp pond throughout the shrimp culture period and to map the composition of microalgae species with the physical and chemical parameters. Based on this study the targeted microalgae species (species which can adapt and also exist in the shrimp pond most frequently throughout a shrimp culture period and also contain high lipid content) will be determined and the data on chemical and physical parameters can be used as a guide for growing the targeted microalgae species in shrimp ponds on a commercial scale.

MATERIALS AND METHODS

Study Site

The study site is a private earthen shrimp farm at Kuala Jalan Bharu, Balik Pulau, Pulau Pinang, Malaysia (5° 21' 13.8" N, 100° 12' 4.2" W). The farm consists of seven tiger shrimp (*Penaeus monodon*) ponds and two white shrimp (*Penaeus vannamei*) ponds. In this study, only one tiger shrimp pond was used. Water supply was from a reservoir located beside the shrimp ponds and the reservoir water was from a sea nearby. The 0.5 hectare pond each has one water inlet connected to the reservoir and one water outlet which was channeled to the ditch for drainage purposes. The pond was stocked with 160000 *P. monodon* shrimp fries [stocking density: >30 post larvae (PL) m⁻², PL 18]. The first water renewal (change) was done at the third week after the shrimps were cultivated and for the rest of the cultivation period the water renewal was performed every two weeks. For water renewal, 30% of shrimp pond water will be discharged into the ditch and the same volume of seawater from the reservoir will be flowed into the pond via the water inlet to replace the water which has flowed out. The shrimp cultivation was performed over a 15 week duration, beginning on 19 February 2008 until 3 June 2008.

Sampling Procedures and Analytical Techniques

The physical [temperature, pH, salinity, light intensity and dissolved oxygen (DO)] and chemical (ammonium; NH₄⁺-N, nitrate; NO₃⁻-N; nitrite; NO₂⁻-N and orthophosphate; PO₄³⁻-P) parameters of the pond water were measured weekly at three different points/spots. All of the physical parameters were measured in situ at around 10 am during sampling time and the readings were taken at 2 different depths, 4 cm from the surface (surface) and at 100 cm from the water surface (bottom). The temperature, pH and DO were determined using a portable meter, YSI model 556 (YSI Incorporated, USA), light intensity was determined by an underwater quantum sensor (model LI-192, LI-COR Bioscience, USA) with data logger light meter (model LI-1400, LI-COR Bioscience, USA), and salinity was determined by using a refractometer (model Milwaukee, AquaCave Incorporated, USA). For the chemical parameters determination, water samples were taken from the surface and at the bottom of the pond at three different points/spots. The water samples were then kept in clean plastic bottles, loaded into a cooler box and transported to the laboratory for: (a) determination of dissolved inorganic nutrients (nitrate, nitrite, ammonium and phosphate)

concentration - 250 ml; and (b) determination of chlorophyll *a* concentration - 500 ml. In the laboratory, the water samples were filtered through 0.4 µm pore size cellulose filter using a pump (B-169 vacuum-system by BÜCHI, Switzerland) for removal of unwanted organisms or other suspended particles. Ammonium determination was performed by Salicylate method, nitrate by cadmium reduction method, nitrite by diazotization method and orthophosphate by ascorbic acid method (Adams 1990). The reagent powder pillows and Hach spectrophotometer 2800 provided by Hach Company (Hach Company 2002) were used in the determination. Weekly rainfall data for the Balik Pulau region was obtained from the Department of Meteorology, Malaysia.

Chlorophyll *a* concentration was measured following the procedure described in Lobban *et al.* (1988) and Jeffrey and Humphrey (1975). Water samples for chlorophyll *a* determination was filtered on the same day of collection. Chlorophyll *a* concentration was determined using the standard spectrophotometric method (APHA 1995) where the absorption of the extract is measured at wavelengths 664 nm and 647 nm.

Microalgae Sampling and Analyses

Microalgae were collected using standard plankton net of 1 m length, 25 cm mouth diameter, and mesh size of 20 µm. The plankton net was towed horizontally and vertically to sample the microalgae. For horizontal towing, plankton sample was collected by lowering the net horizontally into the water then pulled until the net extended and began to tow. The net was scooped through the shrimp pond water while walking slowly along the pond's bank. For vertical towing, the net was lowered into the water to approximately 1 m depth and was kept vertical and off the bottom. The net was pulled straight up through the water column. The samples were then rinsed into collection vessels. Each sampling was divided into 2 clean containers as follows: (a) 100 ml of water containing microalgae for isolating, culturing and maintaining; (b) 100 ml of water containing microalgae was preserved with 2 drops of 1% Lugol's iodine solution for qualitative analysis and microalgae identification and kept at a constant temperature of 26°C. Nearby coastal seawater samples were also taken and brought back to the laboratory in a clean plastic container for microalgae identification. Microalgae identification was based on the morphological characteristics using keys and illustrations by Frank and Terry (1987), Carmelo (1997), Dawes (1998) and Cronberg and Annadotter (2007). Microalgae composition found in shrimp pond were determined (in percentage) according to their taxonomic division.

Statistical Analysis

The data for physical and chemical parameters throughout 15 weeks of the cultivation period were analysed using One-way analysis of variance (ANOVA) by Statistix® 9 (Analytical Software, USA). When significant differences were found, the Tukey method for multiple comparisons among means was applied in order to identify differences between parameters ($p < 0.05$).

RESULTS

Physical Factors

Table 1 shows weekly rainfall data for the Balik Pulau region, and mean concentrations of DO, temperature, pH and salinity of the shrimp pond throughout 15 weeks of the shrimp cultivation period. Figure 1 shows the weekly mean of physical parameters measured at the surface and at the bottom of the shrimp pond during 15 weeks of shrimp cultivation period.

The results of the study showed that the light intensity of the water measured at the surface and at the bottom of the pond ranged from 183.23 ± 3.98 to $1278 \pm 318.34 \mu\text{mol photon m}^{-2}\text{s}^{-1}$ and 68.51 ± 12.11 to $351.91 \pm 134.22 \mu\text{mol photon m}^{-2}\text{s}^{-1}$ respectively [Fig. 1(a)]. Throughout the shrimp cultivation period, the light intensity at the water surface was significantly higher (183.23 to $1278 \mu\text{mol photon m}^{-2}\text{s}^{-1}$) compared to the light intensity at the bottom of the pond (68.51 to $351.91 \mu\text{mol photon m}^{-2}\text{s}^{-1}$). Light intensity at the water surface fluctuated over the cultivation period. The light intensity was higher in week 1 (odd week) but lower in week 2 (even week). This alternating light intensity trend was seen until week 7 but no such trend was seen during the subsequent weeks [Fig. 1(a)].

Water temperature at the surface fluctuated between $29.56 \pm 0.05^\circ\text{C}$ to $31.59 \pm 0.08^\circ\text{C}$ and $29.53 \pm 0.02^\circ\text{C}$ to $31.56 \pm 0.01^\circ\text{C}$ at the bottom of the pond [Fig. 1(b)]. The temperature difference between pond surface and bottom was not significantly different ($p < 0.05$).

DO ranged from 4.56 ± 0.29 to $8.21 \pm 0.71 \text{ mg/l}$ and 4.43 ± 0.07 to $9.30 \pm 0.31 \text{ mg/l}$ at the surface and bottom of the pond respectively [Fig. 1(c)]. The concentration of DO in the water was not significantly different ($p < 0.05$) between the surface and the bottom of the pond except for a few weeks at the final phase of shrimp cultivation (week 8 to 15). During those weeks, DO concentration was higher at the surface than at the bottom of the pond ($p > 0.05$).

The pH of the water in the pond ranged from 7.65 ± 0.01 to 8.5 ± 0.00 and 7.46 ± 0.02 to 8.51 ± 0.08 at the surface and bottom of the pond respectively [Fig. 1(d)]. From week 7 to week 15, pH fluctuation between bottom and surface water was apparent. A lower pH value was detected at the bottom of the pond from week 7 until week 15, with the lowest pH detected in week 8.

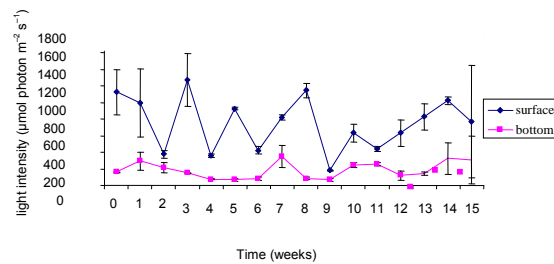
Salinity of the water ranged from $20 \pm 0.0\text{‰}$ to $30 \pm 0.0\text{‰}$ over the cultivation period [Fig. 1(e)]. The salinity of 30‰ was maintained in the early cultivation period from the beginning (week 0) to week 5, but begin to decrease to 25‰ in week 6 to week 8, and decreased further to 20‰ in week 9 until the end of the culture period.

Chemical Parameters

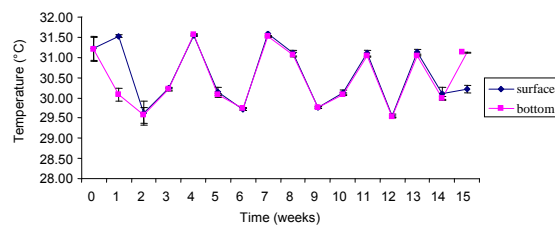
Figure 2 shows weekly mean concentrations of ammonium, nitrate, nitrite and orthophosphate during the 15 weeks of shrimp cultivation period.

Table 1: Weekly rainfall data of Balik Pulau, Pulau Pinang region and mean concentrations of DO, temperature, pH and salinity of shrimp pond throughout a 15 weeks cultivation period.

| Cultivation week | Rainfall (mm) | DO (mg/l) | Temperature (°C) | pH | Salinity (‰) |
|------------------|---------------|-----------|------------------|------|--------------|
| 0 | 0.0 | 4.73 | 31.22 | 8.49 | 30 |
| 1 | 0.0 | 5.18 | 31.53 | 8.01 | 30 |
| 2 | 0.0 | 6.94 | 29.62 | 8.12 | 30 |
| 3 | 0.0 | 7.31 | 30.23 | 8.07 | 30 |
| 4 | 2.0 | 5.69 | 31.55 | 8.06 | 30 |
| 5 | 0.6 | 6.19 | 30.14 | 8.18 | 30 |
| 6 | 4.8 | 5.25 | 29.71 | 8.07 | 25 |
| 7 | 2.8 | 7.19 | 31.59 | 8.20 | 25 |
| 8 | 0.8 | 4.56 | 31.11 | 8.25 | 25 |
| 9 | 10.0 | 8.21 | 29.76 | 8.12 | 20 |
| 10 | 0.2 | 6.34 | 30.13 | 8.5 | 20 |
| 11 | 6.4 | 5.85 | 31.10 | 8.22 | 20 |
| 12 | 0.0 | 6.00 | 29.56 | 8.13 | 20 |
| 13 | 0.2 | 6.14 | 31.14 | 7.95 | 20 |
| 14 | 0.0 | 6.33 | 30.11 | 7.76 | 20 |
| 15 | 0.8 | 6.57 | 30.21 | 7.65 | 20 |

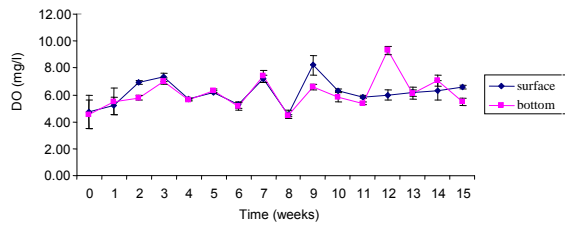


a) Light intensity

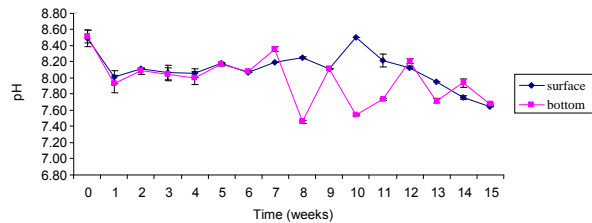


b) Temperature

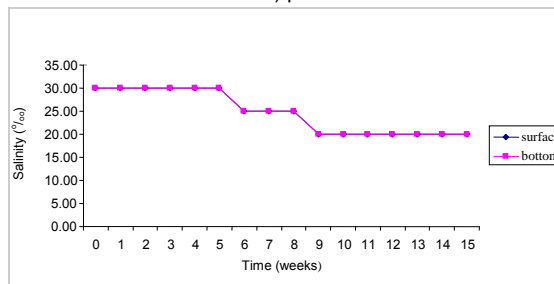
Figure 1: Weekly mean light intensity, pH, DO, temperature and salinity measured at the surface and the bottom of the shrimp pond over 15 weeks of shrimp cultivation period. Error bars represent standard deviation (*continued on next page*).



c) DO



d) pH

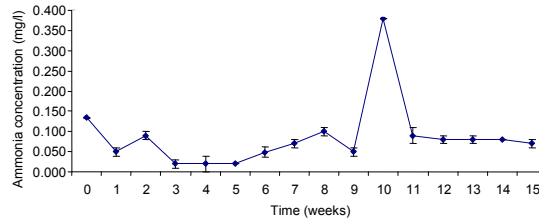


e) Salinity

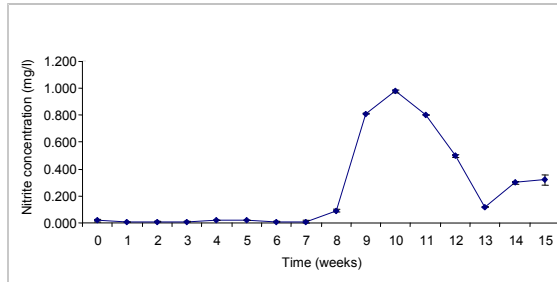
Figure 1: (continued).

Over the cultivation period, mean ammonium concentrations ranged from 0.017 to 0.38 mg/l [Fig. 2(a)]. The ammonium concentrations generally were not fluctuating much from week 1 to week 9. However, a sharp increase in the concentration was observed in week 10 of the cultivation, followed by a decline in the subsequent weeks (weeks 11–15). The mean nitrite and nitrate concentrations ranged from 0.01 ± 0.004 to 0.98 ± 0.01 mg/l and 0.024 ± 0.01 to 2.12 ± 0.035 mg/l respectively [Fig. 2(b) and 2(c)]. The highest nitrate and nitrite concentrations (0.98 mg/l and 2.12 mg/l respectively) were recorded in week 10, and then decreased abruptly in the following weeks. Overall, nitrite and nitrate concentration were below 0.2 mg/l from the early cultivation until week 8 before the concentration increased after week 8 until week 10. The nitrite and nitrate

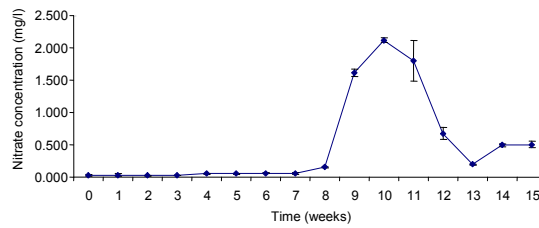
concentrations subsequently declined from week 10 to week 13 and nitrite and nitrate concentrations were again increased slightly in week 14 and 15.



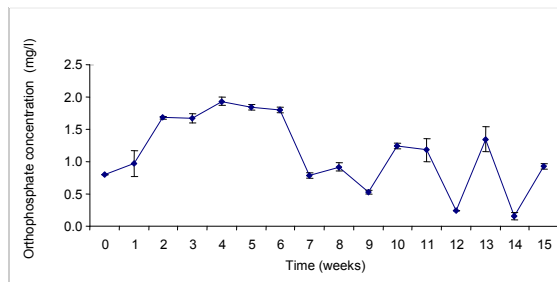
a) Ammonium



b) Nitrite



c) Nitrate



d) Orthophosphate

Figure 2: Weekly mean concentrations of dissolved inorganic nutrients of the shrimp pond over 15 weeks of cultivation period. Error bars represent standard deviation.

Over the study period, mean soluble reactive phosphorus or o-phosphate concentrations ranged from 0.16 ± 0.06 to 1.93 ± 0.064 mg/l [Fig. 2(d)]. During the early stage of the shrimp culture period (week 1 to 5), o-phosphate concentrations were higher compared to the later stage. The concentrations of phosphate were highest at 1.93 mg/l in week 4 and lowest in week 14. The o-phosphate concentrations fluctuated biweekly starting from week 7 until the end of the cultivation period.

Chlorophyll a concentrations in this study ranged from 5.03 ± 2.17 to 32.61 ± 0.35 µg/l (Fig. 3). The highest chlorophyll a concentration was at the beginning of shrimp culture period but the concentration dropped to the lowest value in week 4. Chlorophyll a concentrations then increased slightly from week 5 to week 7 and decreased again from week 8 to week 10. However, chlorophyll a concentrations increased further from week 11 to subsequent weeks until the end of the culture period.

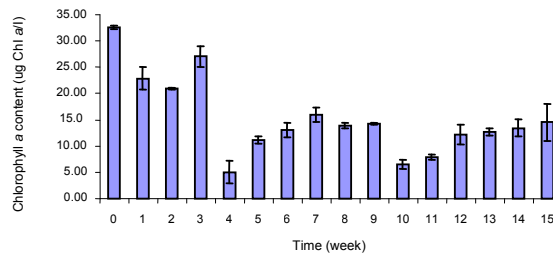


Figure 3: Weekly mean chlorophyll a concentrations in shrimp pond throughout 15 weeks of shrimp cultivation period.

Microalgae Composition

Table 2 shows the microalgae species composition in the shrimp pond throughout 15 weeks of shrimp cultivation period. A total of 19 microalgae species were identified, with Bacillariophyta or diatoms contributing to 72% of the species composition, followed by Chlorophyta (11%) and Cyanophyta or blue green microalgae (11%).

During the first two weeks of the cultivation period when there were no shrimps in the pond, Cyanophyta (blue-green microalgae) dominated the pond followed by Chlorophyta (green microalgae). The Cyanophyta bloom was made up mostly of *Anabaena* spp. and *Oscillatoria* spp. After shrimps were introduced into the pond (week 3), the Bacillariophyta (diatoms) started to appear and then bloomed at the final shrimp culture phase when the salinity was lower (20‰–25‰). Diatoms continued to be the most adaptable group over the period of shrimp cultivation. However, there were a number of times within this culture period when the Chlorophyta overtook the diatoms as a dominant group. The diatom *Cylindrotheca closterium* existed during most of the culture period and can be considered as the most tolerant species in the shrimp pond.

Table 2: Microalgae species composition in shrimp pond during the 15 weeks cultivation period.

| Cultivation period (week) | Microalgae species in the shrimp pond | Dominant microalgae over 15 weeks of cultivation period |
|---------------------------|--|---|
| 0 | <i>Oscillatoria</i> sp., <i>Anabaena</i> sp. | <i>Anabaena</i> sp. (blue green) |
| 1 | <i>Anabaena</i> sp., <i>Chlorella</i> sp., <i>Oscillatoria</i> sp., <i>Navicula</i> sp. | <i>Anabaena</i> sp. (blue green) |
| 2 | <i>Chlorella</i> sp., <i>Cylindrotheca closterium</i> , <i>Amphora</i> sp., <i>Navicula</i> sp. | <i>Chlorella</i> sp. (green) |
| 3 | <i>Amphora</i> sp., <i>Navicula</i> sp., green (<i>Dunaliella</i> sp.), <i>Gyrosigma</i> sp., <i>Nitzschia</i> sp. | <i>Amphora</i> sp. (diatom) |
| 4 | <i>Oscillatoria</i> sp., <i>Chlamydomonas</i> sp., <i>Navicula</i> sp., <i>Chlorella</i> sp., <i>Cylindrotheca closterium</i> | <i>Cylindrotheca closterium</i> (diatom) |
| 5 | <i>Oscillatoria</i> sp., <i>Gyrosigma</i> sp., <i>Nitzschia</i> sp. | <i>Gyrosigma</i> sp. (diatom) |
| 6 | <i>Gyrosigma</i> sp., <i>Navicula</i> sp., <i>Thalassiosira</i> sp. | <i>Gyrosigma</i> sp. (diatom) |
| 7 | <i>Cylindrotheca closterium</i> , <i>Oscillatoria</i> sp., <i>Navicula</i> sp., <i>Thalassiosira</i> sp., <i>Chlorella</i> sp. | <i>Cylindrotheca closterium</i> (diatom) |
| 8 | <i>Gyrosigma</i> sp., <i>Oscillatoria</i> sp. | <i>Gyrosigma</i> sp. (diatom) |
| 9 | <i>Cylindrotheca closterium</i> , <i>Oscillatoria</i> sp. | <i>Cylindrotheca closterium</i> (diatom) |
| 10 | <i>Cylindrotheca closterium</i> , <i>Coscinodiscus</i> sp., <i>Triceratium</i> sp., <i>Cyclotella</i> sp., <i>Suriella</i> sp., <i>Anabaena reniformis</i> | <i>Cylindrotheca closterium</i> (diatom) |
| 11 | <i>Coscinodiscus</i> sp., <i>Cylindrotheca closterium</i> , <i>Navicula</i> sp., <i>Gyrosigma</i> sp., <i>Suriella</i> sp. | <i>Cylindrotheca closterium</i> (diatom) |
| 12 | <i>Cylindrotheca closterium</i> , <i>Gyrosigma</i> sp., <i>Navicula</i> sp., <i>Thalassiosira</i> sp. | <i>Cylindrotheca closterium</i> (diatom) |
| 13 | <i>Chlorella</i> sp., <i>Thalassiosira</i> sp. | <i>Chlorella</i> sp. (green algae) |
| 14 | <i>Chlorella</i> sp., <i>Cylindrotheca closterium</i> , <i>Gyrosigma</i> sp., <i>Pleurosigma</i> sp., <i>Thalassiosira</i> sp. | <i>Gyrosigma</i> sp. (diatom) |
| 15 | <i>Cylindrotheca closterium</i> , <i>Triceratium</i> sp., <i>Thalassiosira</i> sp., <i>Lauderia</i> sp., <i>Coscinodiscus</i> sp. | <i>Cylindrotheca closterium</i> (diatom) |

Microalgae species found in the coastal waters and in the shrimp pond is shown in Table 3. In coastal waters, 80% of microalgae were diatoms and out of this, 38% were not found in the shrimp pond.

DISCUSSION

Physical Factors

In the present study, the amount of light penetration in the shrimp pond was higher at odd weeks and was lower at even weeks. This phenomenon was due to

the water changing regime of the pond. The pond water was changed for the first time in week 3 and the following water changing took place in weeks 5, 7, 9, 11 and 13. The pond water needs to be changed because unchanged water will increase turbidity due to the increase of particulate organic matters. Turbidity will block light penetration resulting in low light intensity. Water changing will bring in clean water resulting in better light penetration into the pond. However, the biweekly fluctuation trend of light intensity in the pond at the early stage of shrimp cultivation does not happen at the final stage. It could be due to the build up of more particulate matters from uneaten feed, dead microalgae and shrimp excretion. According to Guerrero-Galván *et al.* (1999) particulate organic matter and total suspended solid levels were higher during the rainy season, which in this study occurred at the final stage of shrimp cultivation. Delgado *et al.* (2003) also reported that decrease of light penetration into the bottom of the pond was due to heavy precipitation and sludge accumulation.

DO concentration in this study was considered normal and acceptable for a shrimp pond. Cheng *et al.* (2003) reported that DO values higher than 5 mg/l have often been recommended for intensive culture practices. Maintenance of an adequate level of DO in pond water is very important for shrimp survival and prolonged exposure to the stress of low concentration of oxygen can inhibit shrimp growth.

Table 3: Microalgae species found in coastal waters and the shrimp pond.

| Microalgae species | Area | |
|---------------------------------|----------------|-------------|
| | Coastal waters | Shrimp pond |
| <i>Anabaena</i> sp. | √ | √ |
| <i>Bacteriasrium</i> | √ | |
| <i>Bellerochea</i> sp. | √ | |
| <i>Coscinodiscus</i> sp. | √ | √ |
| <i>Cyclotella</i> sp. | √ | √ |
| <i>Cylindrotheca closterium</i> | √ | √ |
| <i>Guinardia</i> sp. | √ | |
| <i>Gyrosigma</i> sp. | √ | √ |
| <i>Lauderia</i> sp. | √ | √ |
| <i>Navicula</i> sp. | √ | √ |
| <i>Odontella</i> sp. | √ | |
| <i>Oscillatoria</i> sp. | √ | √ |
| <i>Pleurosigma</i> sp. | √ | √ |
| <i>Pseudonitzschia</i> sp. | √ | |
| <i>Rhizosolenia</i> sp. | √ | |
| <i>Skeletonema</i> sp. | √ | √ |
| <i>Suriella</i> sp. | √ | |
| <i>Thalassionema</i> sp. | √ | √ |
| <i>Thalassiosira</i> sp. | √ | √ |
| <i>Triceratium</i> sp. | √ | |

Note: √ indicates a presence of particular microalgae in either coastal waters or shrimp pond.

In an aquatic environment such as in shrimp ponds, pH value and CO₂ concentration are controlled by the photosynthetic and respiration processes. CO₂ released by shrimp during respiration will be consumed by microalgae for their photosynthetic process which produce O₂ as a byproduct. The removal of CO₂ through photosynthesis process reduces carbonic acid concentration which will result in the rise of pH in the pond. The low pH value at the bottom of the pond was mainly due to higher sludge accumulation especially during even weeks where the pond water was already two weeks old without changing. With the increase in the amount of sludge, the pH of pond will decrease due to increase in CO₂ concentration as a result of respiration process which occurs in various microorganisms as well as shrimps (Delgado *et al.* 2003).

The decrease in salinity throughout this study was correlated to the evaporation and rainfall. This result was in agreement with the work reported by Guerrero-Galván *et al.* (1999) and Mmochi *et al.* (2002) which found that salinity values were influenced by the evaporation during the hot season and by rainfall in the rainy season. Everett *et al.* (2007) also claimed that their study showed that rainfall dilutes the water column and lowers the salinity.

Chemical Factors

Nutrients such as nitrogen and phosphorus in the shrimp ponds were originated mainly from prepared feed (Páez-Osuna *et al.* 1997; Thakur & Lin 2003; Xia *et al.* 2004; Páez-Osuna & Ruíz-Fernández 2005; Casillas-Hernández *et al.* 2006; Cremen *et al.* 2007), fertiliser used, water pumped into the pond, juveniles stocks, rainfall (Xia *et al.* 2004) and shrimp excretion (Cremen *et al.* 2007). The maximum tolerable concentration of ammonium for shrimp is 0.1 mg/l (Tsai 1989; Anon. 2003). At weeks 0, 8 and 10, ammonium concentrations were higher than the maximum tolerable concentrations; however the concentrations in the other weeks are considered in the acceptable range. Ammonia is toxic to shrimps in high concentrations (Chien 1992). In the present study, ammonium concentrations in the shrimp pond at week 0 (0.134 mg/l), 8 (0.10 mg/l) and 10 (0.38 mg/l) may not be considered a problem because it has been reported that in *P. monodon* growing system, even with frequent water exchange, ammonium concentrations may increase up to 6.5 mg/l (Chen & Tu 1991).

The increase in ammonium concentrations at certain times over the cultivation period could be due to a few reasons; first, when shrimp size increases, the feeding rates will increase accordingly and resulted in increase in ammonium waste (Guerrero-Galván *et al.* 1999). Claybrook (1983) has also reported that ammonium is the major nitrogenous waste excreted by crustaceans. The second reason is the decomposition of organic materials by microbes and fertilisation practices (Smith *et al.* 2002; Tookwinas & Songsangjinda 1999; Guerrero-Galván *et al.* 1999). However, the ammonium concentrations in this study never exceeded 6.5 mg/l (safe concentration to shrimp). This indicates that during the study period the harmful nitrogenous waste was effectively removed by phytoplankton and microbial activity (Shilo & Rimon 1982; Diab & Shilo 1988).

Nitrite concentrations never exceeded the unsafe level of 1.0 mg/l at any time during the study period although there was an abrupt increase in weeks 9

and 10. Nitrate reached a maximum concentration of 2.12 mg/l in week 10 and this concentration is higher than the maximum acceptable concentration of 1.0 mg/l for a shrimp culture pond (Fast & Lester 1992). According to Burford *et al.* (2003), in a shrimp pond with zero-water exchange, nitrate concentrations increased to 10.62 mg/l, which is 80% higher than the present study.

The increase of ammonium, nitrite and nitrate concentrations in week 9, most probably attributed to nitrogen compound that entered the reservoir during heavy rains (week 6 to week 11). According to Xia *et al.* (2004) who studied nitrogen and phosphorus cycling in shrimp ponds, nitrogen input through rainfall was estimated at 10.12 and 8.96 kg ha⁻¹ respectively, due to the differences in rain duration. Furthermore, during rain, surface runoff picks up chemicals such as nitrates and phosphates from adjacent agriculture land and diffused effluent from human domestic litter and transports them to shrimp pond. Ammonium concentrations increased to its highest concentrations in week 10, showing the maximum accumulation of ammonium in the pond. This accumulated ammonium however decreased in the following week most probably due to nitrification process. Nitrification is a transformation process of ammonia (oxidation by bacteria) to nitrite and then to nitrate. From week 11, nitrite and nitrate concentrations also decrease slowly up to week 13 until reaching stable concentrations.

Orthophosphate or soluble reactive phosphorus concentrations were found to be high throughout the cultivation period. This phosphate concentration was higher compared to the concentration reported by Boyd (1990). The study done by Boyd (1990) showed that dissolved orthophosphate concentration were in the range of 0.005–0.02 mg/l, and seldom exceeded 0.1 mg/l even in highly eutrophic water. However, this result is in accordance to that of Fast and Lester (1992) who suggested the tolerable ranges for shrimp culture is <3.0 mg/l. Thakur and Lin (2003) also reported that there was a significantly higher concentration of orthophosphate (0.218–0.384 mg/l) in shrimp ponds with higher stocking density. In zero-water exchange shrimp ponds, the phosphate concentration was high, which ranged from 0.07–1.17 mg/l (Burford *et al.* 2003).

Another probable source of phosphorus other than shrimp feed and shrimp excretion is from the excretion of the zooplankton population. According to Sullivan and Ritacco (1985) and Buttino (1994), some zooplankton species can only tolerate ammonium concentration lower than 0.2 mg/l. Therefore, although the zooplankton population was not quantified in this study, it was assumed that there was an abundance of zooplankton which in turn contributed to the high phosphate concentration because ammonium concentrations in the present study did not reach 0.2 mg/l. Hence, shrimp pond could have higher reactive phosphorus due to zooplankton excretion.

The high concentrations of chlorophyll *a* at the beginning of the cultivation period could coincide with the clear pond water condition and high light intensity. Guerrero-Galván *et al.* (1999) also reported that the production of high algae biomass was driven by optimum environmental factors such as high light intensity and high temperature. In the present study, the pond was irrigated with coastal water and the pond water was fertilised with manure or commercial NPK fertiliser one or two weeks before shrimps were introduced. The fertiliser induced

microalgae bloom in shrimp pond. Pond water with high microalgae density turns into green colour and this is called green water. Green water comprises a high biomass of microalgae mainly blue green and green algae which increase chlorophyll a concentrations in the shrimp pond. After the introduction of shrimps, light penetration into the shrimp pond decreased due to heavy particulate matter from shrimp excretion and uneaten feed. Decrease in light penetration into the pond resulted in the decrease in microalgae biomass as well as the chlorophyll a concentration.

Microalgae Abundance

This study showed that Bacillariophyta, Cyanophyta and Chlorophyta constituted the greatest bulk of the microalgae population in the shrimp pond. In the first two weeks, the pond with no shrimps and fertilised with NPK fertiliser was dominated by Cyanophyta (*Anabaena* spp., *Oscillatoria* spp.), followed by Chlorophyta (*Chlorella* sp.). When shrimps were introduced into the pond, diatoms started to occur and the diatoms became the dominant species during shrimp culture period. According to Smith (1993), accumulated sediments in shrimp ponds consisted mainly of silica and Smith (1994) stressed that amorphous silica is an important component of pond sediment and that the activity of diatoms is fundamental to the silica cycle in shrimp ponds. Hence, an increase in silica content in shrimp pond was suspected in promoting diatom growth which in turn will depress the growth of Cyanophyta especially *Oscillatoria* spp. (Yusoff *et al.* 2002). Microalgae abundance trend in the present study contradicted the study by Cremen *et al.* (2007) which illustrated qualitative and quantitative changes in phytoplankton communities in tropical commercial shrimp ponds using green water with a different stocking densities. According to their study, Chlorophyta is the dominant algae during the initial culture phase (0–35 days of culture), which coincided with high salinity (35.67‰), while the Cyanophyta bloom occurred towards the final culture phase (84–112 or 126 days of culture) when there was low salinity (19.5‰) and a short diatom bloom occurred at the same time.

In the present study, the high phosphate concentrations at the initial phase of shrimp cultivation (week 0 to 5) significantly coincided with the abundance of Cyanophyta. The high nitrate concentrations at midphase (week 6 to 10) and early final phase stimulated diatoms dominance. Vanni and Findlay (1990), Clifford (1992) and Cremen *et al.* (2007) agreed that high phosphate concentrations usually encouraged the growth of Cyanophyta, whereas high nitrate concentration encourages diatoms growth. Cremen *et al.* (2007) revealed that high ammonium and nitrite levels result in high N:P ratio that will promote diatom blooms. In addition, Smith (1983) reported that some shrimp ponds with high nitrogen loading rates could cause the absence or rare occurrence of Cyanophyta.

The microalgae species found in the coastal water were much more diverse compared to the shrimp pond. Although the microalgae in the shrimp pond were originated from the coastal water that was used to irrigate the shrimp pond, a few microalgae species that exist in the coastal water could not be found in the shrimp pond. The absence of some of the coastal water microalgae species in the shrimp pond was most likely due to the fact that the species could

not tolerate to the physicochemical factors of harsh environment. The species that colonised the shrimp pond would be the species that can tolerate and grow in the polluted environment. The present study indicates that Bacillariophyta is the most abundant microalgae in the open sea. Yusoff (2004), however, reported that 73.8% of the microalgae groups in the northern region of the Straits of Malacca were Cyanophyta followed by Bacillariophyceae (diatom) 25.7%, Dinoflagellate 0.5% and Euglenoids 0%.

CONCLUSION

The study showed that different microalgae species tolerate different environmental conditions. Due to the fluctuation in physical and chemical conditions in the shrimp pond, not all species survived throughout the cultivation period. Throughout the 15 weeks of study, the physical and chemical parameters and species abundance were different from time to time. The abundance of certain microalgae at certain time is the indicator of the suitability of the environmental parameter for microalgae growth and survival. The dominant species changed from week to week according to the changes in physical factors and nutrient levels. However, diatom showed dominancy in almost every week during the cultivation period. *C. closterium* was found to be the most tolerant species due to the frequency of its appearance and dominancy in 6 out of 15 weeks of cultivation period.

ACKNOWLEDGEMENT

Funds for this research were provided by the National Oceanography Directorate Division (Ministry of Science Technology and Innovation) through grant no. 304.PJJAUH.650423.D111 to the second author and Universiti Sains Malaysia Fellowship Scheme to the first author. We would like to thank Malaysian Meteorological Department for providing records of meteorological data for the year 2008 and Fisheries Research Institute, Pulau Pinang, Malaysia for providing us research facilities and advices. Special thanks to Mrs. Roziawati binti Mohd Razali, Fisheries Research Officer, Mrs. Loh Siok Lian, the staff of the Biotoxin Marine Laboratory and Ms. Lau Yon Lai, the staff of the Food Chemical Laboratory of the Fisheries Research Institute, Pulau Pinang for their technical support and assistance. We also would like to thank Mr. Lim, the owner of shrimp farm in Kuala Jalan Bharu, Balik Pulau, Pulau Pinang, Malaysia for allowing access to his farm for this study, members of Micro and Macroalgal Culture Laboratory for their kind attention and support, and Ms. Nurita Abu Tahir for helpful comments in improving this manuscript.

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