黒潮圏科学（Kuroshio Science），**5**－2，129－136，2012

Research Paper

Nutrient, phytoplankton and harmful algal blooms in the shrimp culture ponds in Thailand

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Abstract

Relationship between shrimp (black tiger prawn *Penaeus monodon*) production and the dynamics of environmental parameters, in particular, phytoplankton community structure was investigated in three shrimp cultural ponds in the southern part of Thailand in 2007 and 2008. The results showed that the environmental parameters were not so much different among 3 ponds and that concentration of dissolved organic nitrogen and phosphorus were higher than dissolved inorganic nitrogen and dissolved inorganic phosphorus throughout the culturing period. Mean concentration of chlorophyll a was more than 100 μg/l all the ponds. When chlorophyll a concentration increased the total food consumption of shrimp decreased. However, the reduced average daily growth (ADG) of shrimp was observed when dinoflagellate density was high, and ADG was the highest when dinoflagellate density decreased. On the contrary, in the pond where diatom, cyanobacteria and green algae were predominated, relatively high values in ADG and the shown throughout the culturing period. These results indicate that shrimp growth, survival rate and net production were negatively affected by dinoflagellate blooming.

Key words: Nutrient, dinoflagellate, phytoplankton, shrimp production, Thailand

Introduction

Shrimp aquaculture is one of the developing economic activities in the Asia-Pacific region, where about 80% of cultured shrimp was produced in the world (Trott and Along, 2000; Wolanski *et al.,* 2000). Extensive shrimp culture in Thailand started around 1930, and commercial shrimp hatchery was established in 1974. Intensive shrimp culture has promoted Thailand to be the largest shrimp exporter of the world since 1985 (Richardson, 1997). Intensive shrimp aquaculture system requires high protein in order to maximize the production per unit area (Kureshy and Davis, 2002). Feed in shrimp pond is typically enriched with both organic and inorganic nutrients. Water quality depends on the management of shrimp farming practice, *i.e.* stocking density of shrimp, water supply the amount and quality of feed and the use of fertilizers (Songsangjinda *et al.,* 2006). Organic matters in uneaten feed, solubilized protein and waste metabolizes such as ammonia are usually accumulated in the water column and bottom sediment of shrimp ponds and resulting in eutrophication in surrounding aquatic environment (Burford and Williams, 2001; Songsangjinda *et al.,* 2004). Several reports have indicated that the eutrophication of shrimp culture ponds with organic matters and nutrients resulted in the blooming of variable phytoplankton community in the ponds. (Alonso and Osuna, 2003; Songsangjinda, 1994).

Occurrences of the blooming of phytoplankton often results in farm industry in shrimp mortality and economic loss (Alonso and Osuna, 2003). Many reports showed mass mortality by phytoplankton blooming many regions in the word. For example, the blooming of dinoflagellates *Alexandrium tamarense* caused mortality of *Penaeus monodon* in Taiwan (Huei *et al.,* 1993). Similarly dinoflagellate *Gyrodinium instriatum* in Ecuador and the cyanobacteria *Synechocystis diplococcus, Schizothrixcal cicola,* and the dinoflagellate *Prorocentrum minimum* also caused mortality of shrimp in NW Mexico (Alonso and Osuna, 2003). These facts suggest that the blooming

Received 12 May 2011; accepted 29 February 2012.
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of phytoplankton reduces in feeding and growth of shrimp and disease susceptible increases. The management of shrimp feeding and water and sediment qualities are important to maintain phytoplankton community structure (Strickland and Parsons, 1972). However, there are further few studies on the influence of phytoplankton growth on the shrimp condition in Thailand.

Aims of this study are to investigate the variations of physico-chemical parameters, and chlorophyll a with respect to food intake of the black tiger prawn *P. monodon,* and to evaluate the fluctuation and effects of different phytoplankton group on shrimp condition in order to get important information on predicting and successful management for shrimp culturing.

Material and methods

Study area and pond management

The shrimps farm investigated in this study are located in Songkhla province, the southern Thailand. Shrimp culture techniques were determined from the degree of pond management applied throughout the production cycle starting from the first day of pond preparation to the last day of shrimp harvest. The post larvae (PL) 18 of *P. monodon* with the average size about 15-18 mm were stocked at the density of 15-20 PL/m^2. The dissolved oxygen (DO) was supplied by using aerator. Farmers' records were used to quantify the amounts of manure, fertilizers, and feed used on the basis of day to day management practices. The production of shrimp was also recorded after shrimp harvest. The detailed information on farm practices, such as farm husbandry and shrimp health management practice were interviewed and recorded.

Physico- chemical parameters analyses

Experiments were carried out in three shrimp ponds (Ponds 1 and 2: July to August, 2007 and Pond 3: June to July, 2008) on a weekly basis. Water temperature and dissolved oxygen (DO) were measured using a DO-meter (YSI 158). Water pH was measured using a pH meter (WTW, model 330) and water transparency was measured using a secchi disc. Salinity was measured with a salinometer (ATAGO S/Mill-E) and expressed as a unit of part per thousand (ppt).

Dissolved and particulate matters analyses

Water samples were collected from 30 cm depth every week at 5 locations inside the ponds and one litter of water from each location was mixed (composite sample method). The mixed water samples were filtered through either 47 mm Whatman GF/C or GF/F glass fiber filters. The samples were then transported to the laboratory for analyses. Filtrates were analysed for the concentrations of nitrate-nitrogen (NO_3–N), nitrite-nitrogen (NO_2–N), ammonia-nitrogen (TAN), and dissolved inorganic phosphorus (DIP) using an automatic analyzer (Bran+Luebbe, TRAACS 800). Dissolved inorganic nitrogen (DIN) was calculated by the total of NO_3–N, NO_2–N and TAN.

The concentration of total dissolved nitrogen (TDN) was determined using the method described by Nydahl (1978), in which all forms of nitrogen in filtered water samples were oxidized to nitrate by pyrox-oxidixing and were determined using TRAACS 800. The concentration of dissolved organic nitrogen (DON) was calculated by subtracting DIN from TDN. Total dissolved phosphorus (TDP) was estimated after conversion of all forms of dissolved phosphorus to orthophosphate using pyrox-oxidixing and also determined with TRAACS 800. Dissolved organic phosphorus (DOP) concentration was obtained by subtracting DIP from TDP. The same filtrate samples were also used for analyzing the dissolved organic carbon (DOC) with a TOC analyzer (SHIMADZU, TOC-5000A).

Amount of total suspended solids (TSS, mg/l) was determined using the methods of gravitation method. A known volume of water was filtered onto a pre-weighed and pre-dried (110 °C for 24 h) Whatman GF/F glass fiber filter. The filter was then dried at 80 °C for 24 h and the weight of total suspended solids was calculated from the difference between the initial and final weights (Clesceri ***et al.,*** 1989; Strickland and Parsons, 1972).

For analyses of particulate organic carbon (POC) and particulate organic nitrogen (PON), a portion of each sample was filtered through a 25 mm Whatman GF/F glass fiber filter (precombusted at 500 °C for 2 hrs). Filters were immediately frozen and kept under -30 °C until analysis. Before the analysis the filters were dried over night at 90 °C and then were analyzed for C and N contents using the high temperature combustion with a CHN analyzer (LECO, CHN-900).

Chlorophyll a and predominant phytoplankton analyses

Water samples were filtered through 0.45 μm cellulose acetate filter papers for determining content of chlorophyll a. Filter samples were extracted over night using 90% acetone, then the clear extracted solutions were measured using spectrophotometric method and calculated the concentration of chlorophyll a (chl. a) according to the equation described in Clesceri *et al.* (1989).

Qualitative and quantitative analyses of phytoplankton were carried out on the basis of weekly intervals. Two litter of phytoplankton samples were collected from the ponds by water sampler and filtrated through fine-meshed (25 μm) plankton net. The samples trapped on the net were immediately preserved with 0.7% Lugol's solution in plastic bottles (APHA *et al.,* 1995). Phytoplankton density was estimated using a sub-sampling technique and Sedgwick–Rafter (S–R) cells under microscope. Cell density of phytoplankton was counted using the formula proposed by Yamiji (1980) and Wongrat (2001) and were expressed as the number of cells per ml of water. Taxonomic community structures of phytoplankton were identified in a general level. The relationship between phytoplankton (chlorophyll a and dinoflagelate density) and shrimp conditions (total food consumption and average daily growth) were performed using linear regression analysis with add-on statistical software for Microsoft Excel ($P<0.05$).

Total food consumption, food conversion ratio (FCR) and average daily growth (ADG)

Shrimps were fed with commercial shrimp feed (crude protein content 42%, C.P. Shrimp feed No. 1-3) at 3 or 4 times a day. The amount of feed taken up by shrimp in each meal was recorded and calculated as total food consumption per day. FCR was calculated during shrimp harvesting by dividing total food consumption by total shrimp production. About 250-500 shrimps were collected and their body weights were measured for calculating the average growth of shrimp. Average daily growth (ADG; g/shrimp/day) was calculated from the daily body weight change within a period of time as the following equation

ADG = BW2 – BW1/ (T2-T1)

BW1 and BW2 = Average of Shrimp body weight (g) of day 1 and day 2

T1 and T2 = Time of shrimp culturing of day 1 and day 2

Results

Growth, survival and production of shrimp

Results of shrimp performance indices are presented in Table 1. Total periods of shrimp culturing were 207, 181 and 139 days in Pond 1, Pond 2 and Pond 3, respectively. Average net production, final body weight, and percentage of shrimp survival were highest in Pond 2, and were 0.56 kg/m^2, 34.5 g/shrimp/day and 83%, respectively. In Pond 3, although FCR was the lowest (1.88), net production (0.32 kg/m^2) and percentage of shrimp survival (63%) were lower than those in Ponds 1 and 2.

Table 1. Shrimp growth and production in three ponds.

Variable	Pond 1	Pond 2	Pond 3
Total culturing (days)	207	181	139
Net production (kg/m^2)	0.47	0.56	0.32
Final weight gain (g/shrimp)	28.6	34.5	33.3
Survival rate (%)	80	83	63
Food conversion ratio (FCR)	2.58	2.13	1.88

Water qualities

Averages of DO concentration during the period of culture were 5.9-7.5 mg/l and never decreased less than 4 mg/l in all ponds (Table 2). Mean values of pH, salinity, and temperature in three ponds were 7.9-8.3, 20-24 ppt, and 29.2-29.7 °C respectively, and differences of such environmental parameters among three ponds were small. Averages of transparency and total suspended solids (TSS) were 19-48 cm and 95-117 mg/l, respectively. However, lower transparency and higher TSS values were obtained in Pond 2 (Table 2). Range of water depth of three ponds was 100-120 cm. Transparency was higher in the beginning of the culture period and slightly declined during culturing period in all ponds.

Mean values of the concentration of nutrient and organic matter are shown in Table 3. The mean concentrations of DOC in Ponds 1 (901 μM) and 2 (1350 μM) were higher than in Pond 3 (237 μM). Average DON and DOP in Ponds 2 and 3 were higher than DIN and DIP throughout the culturing period. The results showed that most of dissolved inorganic nitrogen was NH4-N, but most of nitrogen was DON and PON (Table 3). Concentrations of DIN, DON, DIP and DOP in Pond 1 (Fig. 1 a and d) and Pond 2 (Fig. 1 b and e) slightly fluctuated over the culturing period.

Phytoplankton and shrimp condition

Table 2. Mean values and ranges of water quality factors in three shrimp ponds.

General parameters	Pond 1	Pond 2	Pond 3
	Mean ± SD	Mean ± SD	Mean ± SD
DO (mg/l)	5.9±1.3	5.8±0.7	6.9±0.5
pH	7.9±0.4	8.1±0.3	8.0±0.1
Salinity (ppt)	19.8±1.2	23.3±2.1	23.7±1.8
Temperature (°C)	29.3±1.1	29.7±0.9	29.2±0.4
Transparency (cm)	29.3±1.1	18.6±16.8	48.3±4.1
TSS (mg/l)	94.7±31.3	117±62.0	94.7±31.3

Table 3. Mean values of inorganic nutrient and organic matter concentrations in three shrimp ponds.

Nutrients (µM)	Pond 1	Pond 2	Pond 3
	Mean	Mean	Mean
DOC	901	1350	237
POC	825	1360	583
NH4-N	13.7	22.0	4.8
DIN	17.4	24.7	6.9
DON	125	111	107
PON	138	229	121
DIP	2.39	0.48	0.87
DOP	2.41	3.26	6.96

Fluctuations and relationship between chlorophyll a concentration and total food consumption of shrimp in Ponds 1, 2 and 3 are shown in Fig. 2 a, b and c, and Fig. 3 a,b, and c, respectively. There was a negative ($P<0.05$) linear relationship between chlorophyll a concentration and total food consumption of shrimp (Fig. 3 a-c). In pond 1, chlorophyll a ranged between 60 µg/l as the minimal value on the 99th culturing day to 312 µg/l as the maximal value on the 113th day, which reversely corresponded to changes in total food consumption. In particular, on the 113th day, along with the abrupt increase in chlorophyll a concentration, total food consumption decreased drastically to 38 kg/day on that day (Fig. 2 a and Fig. 3 a). In Pond 2, the total food consumption was relatively high and stable, but it also seems that chlorophyll a concentration and total food consumption showed reverse relationship (Fig.2 b and Fig. 3 b). In Pond 3, fluctuations of chlorophyll a and total food consumption showed similar reverse tendency to that in Pond 1 (Fig. 2 c and Fig. 3 c).

Variation of phytoplankton communities in 3 ponds are shown as four main taxonomic groups; diatom, dinoflagellate, cyanobacteria and green algae (Fig. 4). In Ponds 1 and 2, predominant phytoplankton was diatom (Fig. 4 a, b), mainly *Coscinodiscus* sp., *Rhizosolenia* sp., *Guinardia* sp., *Skeletonema* sp., *Navicula* sp., *Prorosigma* sp. and *Gyrosigma* sp, while in Pond 3, it changed from dinoflagellate to diatom, followed by cyanobacteria (Fig. 4 c). When we considered the variation of dinoflagellate density and ADG of shrimp, average ADGs in Pond 1(low dinoflagellate density) and Pond 2 (no dinoflagellate cell) were 0.2 g/day/shrimp and 0.27 g/ day/shrimp, respectively, and it were higher than those in Pond 3 (Fig. 5 a-c) throughout the period of shrimp culture. In contrast, the predominant phyto-

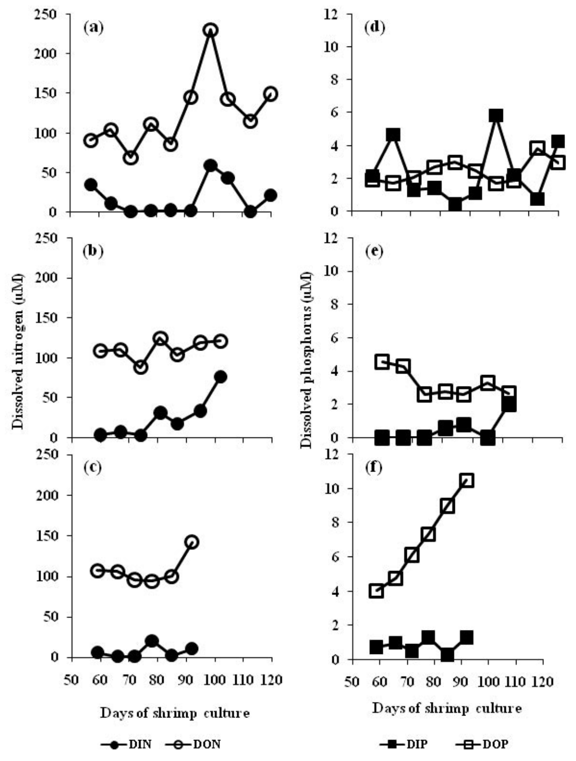

Fig. 1. Fluctuations of dissolved inorganic nitrogen (DIN), dissolved organic nitrogen (DON) in Pond 1 (a), Pond 2 (b) and Pond 3 (c), and dissolved inorganic phosphorus (DIP) and dissolved organic phosphorus (DOP) in Pond 1 (d), Pond 2 (e) and Pond 3 (f), respectively.

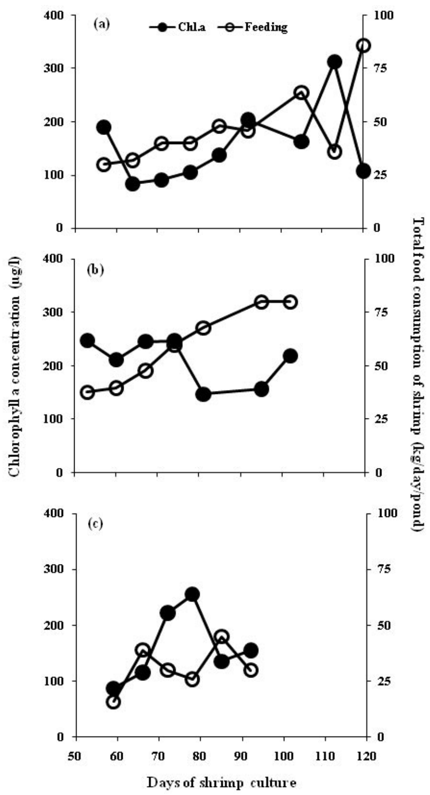

Fig. 2. Fluctuations of chlorophyll a concentration and total food consumption during shrimp culture in Pond 1 (a), Pond 2 (b) and Pond 3 (c), respectively.

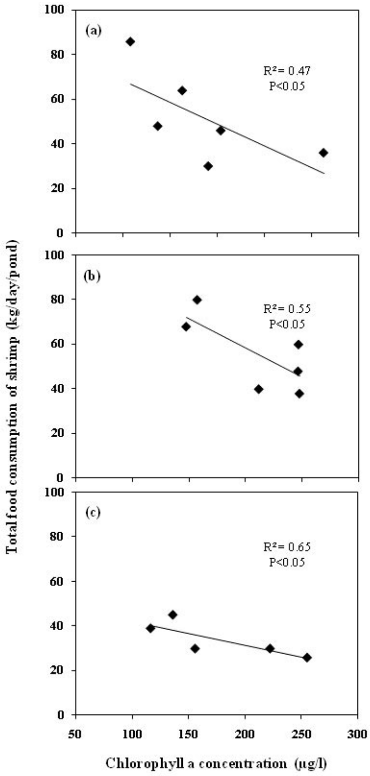

Fig. 3. Relationship between total food consumption of shrimp (kg/day/pond) and the concentration of chlorophyll a (µg/l) in Pond 1 (a), Pond 2 (b) and Pond 3 (c), respectively.

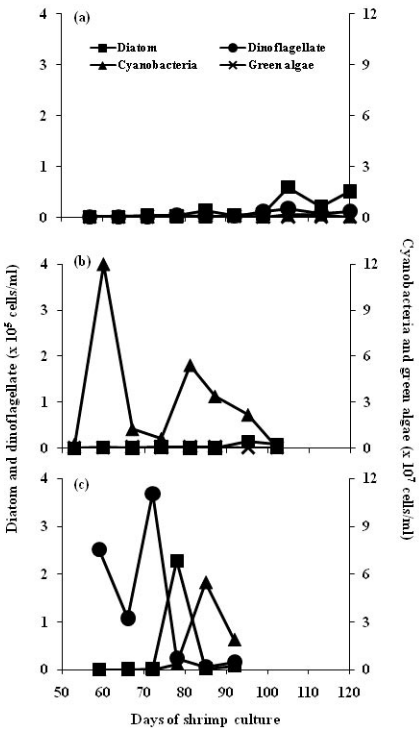

Fig. 4. Fluctuations of phytoplankton cell density (cells/ml) of dinoflagellates, diatom, cyanobacteria and green algae in Pond 1 (a), Pond 2 (b) and Pond 3 (c), respectively.

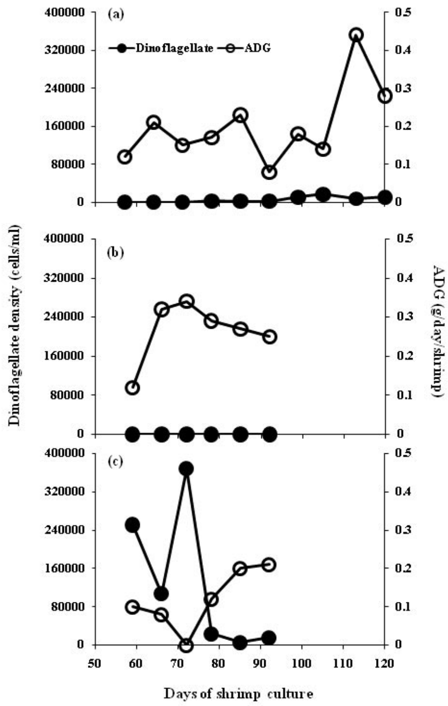

Fig. 5. Changes in dinoflagellate cell density and average daily growth (ADG) of shrimp in three shrimp ponds; low (a: Pond 1), not detected (b: Pond 2) and high (c: Pond 3) densities of dinoflagellate, respectively.

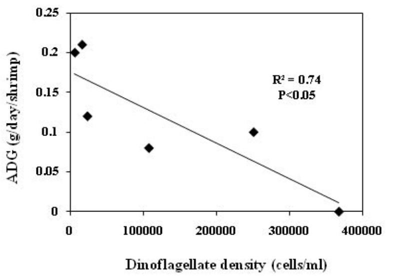

Fig. 6. Relationship between average daily growth of shrimp (ADG; g/day/shrimp) and dinoflagellate density (cells/ml) in pond 3.

plankton community in Pond 3 was dinoflagellate on the 60th-80th day of culturing and then shifted to diatom and cyanobacteria (Fig. 4 c). When dinoflagellate was predominated and in high density (~ $3.68{\times}10^5$ cells/ml) on the 72nd culturing day, shrimp ADG was lower than 0.1 g/ day/shrimp, but it increased when dinoflagellate decreased or disappeared (Fig. 5 c). In addition, in Pond 3, the amount of dinoflagellate densities showed reverse relationship to shrimp growth ($P<0.05$) (Fig. 6).

Discussion

This study was carried out to understand the environmental conditions including physico-chemical and biological (chlorophyll a and phytoplankton community) parameters and their effects on the physiology of shrimp in culture ponds of a black tiger prawn *P. monodon* in 2 occasions. Results indicate that during the culture period, DO concentrations in each pond were stable due to using aerator to maintain the concentration to be above 4 mg/l, which is suitable for shrimp and this promotes good shrimp growth (Songsangjinda *et al.,* 2000). Results of the fluctuation of nutrients (Fig. 1) indicate that the dissolved nitrogen concentration in water was lower than that of particulate nitrogen (Table 3). This suggests that a large portion of nitrogen was in the form of suspended solids.

In the present study, concentrations of chlorophyll a were usually more than 100 µg/l in all ponds (Fig. 2). This was controversy with the fluctuations of dissolved inorganic nutrient concentration (Fig. 1), which may be attributed to that phytoplankton could take up dissolved nutrients and grew in the pond (Krom and Neori, 1989). The results clearly showed that nutrient variations were related to the phytoplankton growth (Fig. 1-2). The main source of nutrients loaded to the shrimp pond is the shrimp feed. This suggests that the pellet feed may properly stimulate phytoplankton growth in the shrimp pond.

From results obtained in this study, shrimp growth depended not only on stocking density and feeding of shrimp itself and water quality but also on the phytoplankton biomass and community structure (Hallegraeff, 1993; Piehler *et al.,* 2004; Richardson, 1997). Results of fluctuation of chlorophyll a, total food consumption and phytoplankton community structure indicate that when the chlorophyll a concentration increased, total food consumption of shrimp decreased (Fig. 2 a-c and Fig. 3 a-c). In particular, when the predominant were dinoflagellates *Ceratium* sp. and *Gymnodinium* sp. in Pond 3, shrimp growth and production decreased (Fig. 5 c and Table 1). A previous report showed that toxic dinoflagellate, *Gymnodinium* spp. caused a loss in shrimp aquaculture in China (Jiasheng *et al.,* 1993) and a massive fish kills by a *Ceratium fusus* in Kagoshima Bay, Japan (Onoue, 1990). In the present study, the environmental parameters were not so much different in all ponds (Table 3), but only predominant phytoplankton was different (Fig. 4). Result indicated that shrimp growth, survival rate and net production of shrimp were seriously impacted by occurring of dinoflagellate in the shrimp culturing period and it resulted in reducing the survival rate and loss of shrimp production (Alonso and Osuna, 2003; Hallegraeff, 1993; Songsangjinda *et al.,* 2006).

In conclusion, high concentration of chlorophyll a impacted on shrimp physiology as decreasing food uptake and the consequent reduction of total food consumption. The occurrence of dinoflagellate, in particular, in the shrimp culture pond gave significant negative effects on the shrimp growth and production. We have to clarify the mechanism of bad effects on shrimp by dinoflagellate in the future.

Acknowledgements

This study was supported by the Ministry of Education, Culture, Sports, Science, and Technology (Monbukagakusho), Japanese Government, JSPS, and the Fund of the President of Kochi University. We are thankful to the staffs of Dumrong farm for their valuable helps with field work and collecting samples.

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タイ国のエビ養殖池における栄養塩と植物プランクトンの変動および有害プランクトンの増殖

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要　旨

タイ南部の３つのエビ養殖池において、2007年から2008年にかけて、ブラックタイガー（*Penaeus monodon*）の成長・生産と種々の環境要因、特に植物プランクトンの群集組成との関連について調べた。溶存態有機窒素および同リン濃度は無機態窒素・リンよりそれぞれ高く、クロロフィルa濃度は常に100µg/l 以上であった。この傾向はいずれの養殖池でもそれほど大きな差異は見られなかった。また環境中のクロロフィルa濃度が増加すると*P. monodon*の摂餌量が減少した。中でも渦鞭毛藻の分布密度が増加すると*P. monodon*の日間成長率（ADG）が低下し、逆に同藻の分布密度が減少するとADGが上昇した。一方、植物プランクトンの優占種が珪藻類・ラン藻類・緑藻類など渦鞭毛藻以外であった養殖池では、*P. monodon*のADGは飼育期間を通して比較的高い値に保たれていた。これらの結果から、*P. monodon*の成長、純生産量、生存率等はいずれも渦鞭毛藻の増殖により悪影響を受けていることが明らかとなった。

キーワード：栄養塩、渦鞭毛藻、植物プランクトン、エビ養殖、タイ国