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PROOF
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Annual Variation Characteristics of Chlorophyll a in Typical Sea Cucumber *Apostichopus japonicus* Culture Ponds

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Abstract

The annual variation features of chlorophyll a in *Apostichopus japonicus* culture ponds were investigated in this study. CHL-A concentration presented clear seasonal fluctuations in the ponds with annual mean value of 19.60 ± 11.29 µg L^{-1}, in which it showed peaking value in summer time. The contributions of size-fractionated phytoplanktons to CHL-A were ordered as micro > nano > meso–macro > pico. The combined influences of SiO_3^{2-}-Si, NO_2^{-}-N, salinity and water temperature on CHL-A concentrations in the ponds were extremely significant ($F > F_{0.01}$), in which SiO_3^{2-}-Si concentration was the controller among them.

Introduction

Chlorophyll a (CHL-A) is a photosynthetic pigment present in all species of phytoplankton including algae and cyanobacteria (Gregor & Maršálek, 2004). As a reliable index, CHL-A has been widely used for assessing phytoplankton biomass and primary productivity (Lloyd & Tucker, 1988; Boyer, Kelble, Ortner, & Rudnick, 2009). Phytoplankton as the primary producer in aquatic ecosystem affects the abundance and diversity of aquatic organisms, and set the upper limits to fishery yields (Chassot *et al.*, 2010). CHL-A concentration and size-fraction can reflect the biomass level, size distribution and community structure characteristics of phytoplankton. Along with the development of aquaculture, monitoring and maintenance of CHL-A in cultured water have become more and more important (Wang, Pu, & Sun, 2016).

Sea cucumber, *Apostichopus japonicus*, as a deposit feeder, is found along the coast of northwestern Pacific Ocean, including the coastal areas of Far East of Russia, Japan, South Korea, Bohai Sea and Yellow Sea of China (Sloan, 1984). In recent years, sea cucumber culture has become one of the most profitable aquaculture industries in China, and yielded 204 359 tons with value exceeded 87.5 billion US dollars in 2016 (Ministry of Agriculture of China, 2017). Presently, *Apostichopus japonicus* is mainly cultured in coastal ponds in China (Ren *et al.*, 2010).

Studies on CHL-A have been conducted in various water bodies from coastal estuarine areas to oceanic, mainly focusing on the spatial and temporal changes, size distribution, and relationships with environmental factors (Iriarte & Purdie, 1994; Agawin, Duarte, & Agustí, 2000; Calvo-Díaz, Morán, & Suárez, 2008; Azhikodan & Yokoyama 2016). However, presently there are still limited information on the CHL-A in sea cucumber culture pond. Especially, there are lack of reports on the relationship between CHL-A and ecological factors in *Apostichopus japonicus* culture pond. The aim of this study was to reveal the annual variation rules of CHL-A concentration and size-fractionated structure, the influence of main ecological factors on CHL-A in the culture pond of *Apostichopus japonicus*; to provide scientific supports for technique optimization in sustainable management of sea cucumber culture pond.

Materials and Methods

The experiment was conducted in typical sea cucumber culture ponds located in Dalian, Liaoning Province, China (39°38'N, 122°57'E). Three representative ponds (numbered as 3, 4 and 9, respectively), with mean area of 3.02 hm^2 and mean water depth of 1.79 m, were investigated from October 2005 to October 2006. The water in the ponds was exchanged semi-monthly in summer and autumn, but monthly in winter and spring according to the rhythm of the tides. The basic culture condition and water quality of the ponds are shown in Table 1. There were five sample locations set in each pond, where sea water was sampled using a water sampler (WB-PM, Beijing) from upper, middle and lower water and mixed as one at each sampling location. Water samples for analysis of CHL-A and environmental factors were sampled before water exchange each time, in which the sampling periods were semi-monthly in summer and autumn and monthly in winter and spring. For the determination of CHL-A concentration, water samples of 500 ml were collected and filtered through a 0.45 µm Waterman GF/F membrane, and the CHL-A concentration was determined using fluorospectrophotometry according to the national specification for marine monitoring of China (GB 17378.7). CHL-A size-fraction was graded by filtering seawater through a series of sieves, namely membrane of 200 µm, 20 µm, 2 µm, and 0.2 µm. The CHL-A contents of size-fractionated phytoplankton (meso–macro: >200 µm, micro: 20–200 µm, nano: 2–20 µm, pico: 0.2–2 µm) were determined in sequence and finally calculated using subtraction method. Water temperature, salinity, pH value and dissolved oxygen were measured with multi-parameter water quality analyzer (Model 556, Yellow Springs Instruments, USA). Transparency was measured using a Secchi Disc. Nutrient concentrations (NH_4^+-N, NO_3^--N, NO_2^--N, PO_4^{3-}-P and SiO_3^{2-}-Si) were measured according to the national specifications for the oceanographic survey of China (GB/T 12763.4).

Statistical Analysis

Regression analyses with critical probability levels of 0.05 or 0.01 were used to evaluate the correlations between CHL-A and ecological factors. Monadic regressive analyses were used to define the correlations of CHL-A with ecological factors. Stepwise multiple regression analysis (SMRA) was used to evaluate the combined influence of ecological factors on CHL-A level, and the partial correlation coefficients (PCC) revealed the order of role played by each ecological factor in it. SPSS Statistics v.23 was performed for statistical analyses.

Results

The CHL-A concentration presented obvious seasonal fluctuation during the experimental period with annual mean value of 19.60±11.29 µg L^{-1} in the

Table 1. Cultured conditions and ecological parameters (mean±SD) in the investigated *Apostichopus japonicus* culture ponds

Ponds	NO. 3	NO.4	NO.9
Area (hm^2)	2.67	3.05	3.33
Water Depth (m)	1.74	1.80	1.82
Seeding Density (ind. hm^{-2})	56620	44570	48298
Seeding Size (g $ind.^{-1}$)	1.33	1.31	1.11
Density of Settlement Substrata (ind. hm^{-2})	2222	2222	2222
Water temperature (ºC)	-1.42 - 26.54	-1.40 - 26.52	-1.43 - 26.91
Salinity	31.14±3.00	31.12±3.16	30.59±2.84
pH value	7.89±0.16	8.04±0.13	7.90±0.19
Dissolved Oxygen (mgO_2 L^{-1})	8.24±1.36	8.52±1.59	8.44±1.09
Transparency (m)	1.02±0.46	1.06±0.50	0.85±0.55
NH_4^+-N (mg L^{-1})	0.0514±0.0375	0.0512±0.0399	0.0397±0.0262
NO_3^--N (mg L^{-1})	0.1811±0.1513	0.1676±0.1308	0.1810±0.1292
NO_2^--N (mg L^{-1})	0.0250±0.0269	0.0247±0.0390	0.0131±0.0142
PO_4^{3-}-P (mg L^{-1})	0.0116±0.0050	0.0149±0.0109	0.0113±0.0061
SiO_3^{2-}-Si (mg L^{-1})	0.0202±0.0115	0.0183±0.0124	0.0208±0.0135

There were no significant differences in the ecological parameters among the three culture ponds (one-way ANOVA followed by Duncan's test, P>0.05).

investigated ponds. The CHL-A level showed a general declined trend in autumn, winter and spring, but had an increased trend in summer, and finally peaked in August (Figure 1). The mean value of CHLA concentration in each season was 23.51 μg L^{-1} (in autumn), 11.14 μg L^{-1} (in winter), 10.86 μg L^{-1} (in spring) and 25.76 μg L^{-1} (in summer), respectively, and there were no significant differences among the three investigated ponds (P>0.05).

The size-fractionated structure of CHL-A and its seasonal variations in *Apostichopus japonicus* culture ponds are shown in Figure 2. Microphytoplankton was the dominant contributor to CHL-A throughout the year with the highest percentage in autumn (48.60 %) and lowest percentage in summer (39.22 %). There were no significant correlations among microphytoplankton percentage, water temperature and salinity ($F < F_{0.05}$). The minimum contribution to CHL-A was from meso–macrophytoplankton except winter. In winter, the smallest contribution came from picophytoplankton. The contribution of size-fractionated phytoplankton to CHL-A in *Apostichopus japonicus* ponds were ordered as micro (44.40%) > nano (27.32%) > meso–macro (14.39%) > pico (14.02%).

The correlation between CHL-A and main ecological factors in *Apostichopus japonicus* culture pond is shown in Table 2. The CHL-A concentration had extremely significant correlations with water temperature, salinity, SiO_3^{2-}-Si content, respectively ($F > F_{0.01}$), meanwhile, its level also showed significant correlations with transparency, NO_3^--N and NO_2^--N contents in the ponds, respectively ($F > F_{0.05}$). The stepwise multiple regression analysis (SMRA) was used to further evaluate the comprehensive influence of ecological factors on the CHL-A level, and the final regression equation was: CHL-A = 35.412 + 0.124T + 0.295Si − 0.804S + $0.074NO_2^-$ (n = 57, R = 0.667, $F > F_{0.01}$), where CHL-A, T, Si, S and NO_2^- meant CHL-A concentration (μg L^{-1}), water temperature (°C), SiO_3^{2-}-Si concentration (μg L^{-1}), salinity and NO_2^--N concentration (μg L^{-1}), respectively. According to partial correlation coefficients (PCC), the effects of the factors in the equation on CHL-A concentration were ordered as SiO_3^{2-}-Si (0.256) > NO_2^--N (0.231) > Salinity (−0.182) > Water temperature (0.085).

Discussion

The CHL-A concentration is commonly used to reflect phytoplankton biomass, and it is also considered the principal variable to use as a water trophic state indicator (Boyer et al., 2009). The annual mean CHL-A concentration in our study was higher than that in some natural freshwater lakes and coastal waters, such as Lake Neusiedlersee (5.06 μg L^{-1}) in central Europe, Lake Erie (<10 μg L^{-1}) in North America and the coastal water of Southampton (1.25 μg L^{-1}) in England; at the same level as in Lake Taihu (20.07 μg L^{-1}) in eastern China; and lower than in *Ictalurus punctatus* (341.0 μg L^{-1}) and *Litopenaeus vannamei* (105.0 μg L^{-1}) culture ponds (Lloyd & Tucker, 1988; Vörös & Padisák, 1991; Iriarte & Purdie, 1994; Witter, Ortiz, Palm, Heath, & Budd, 2009; Jiang *et al.*, 2010; Zhang *et al.*, 2016). The investigated ponds can be classified as eutrophic based on the classifications of CHL-A index values of different trophic state waters (United States Environmental Protection Agency, 1979). This eutrophication state of *Apostichopus japonicus* culture pond might be related with the trophic state of the surrounding sea area and the adjacent freshwater rivers. The seasonal variation data of CHL-A concentration was obviously observed in *Apostichopus japonicus* culture pond, the values in summer and autumn were higher than in other seasons, which might be the result of the combined effects of water temperature and nutrients.

The data of size-fractionated CHL-A showed the micros dominated the phytoplankton community in this study. In general, small phytoplankton (such as nano and

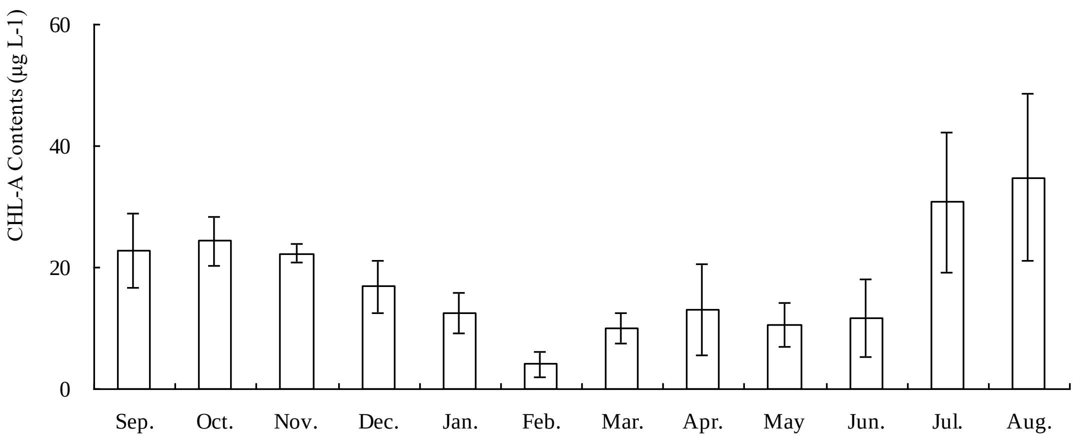

Figure 1. Monthly variations of chlorophyll a concentration (means ± SD) in the investigated *Apostichopus japonicus* culture ponds.

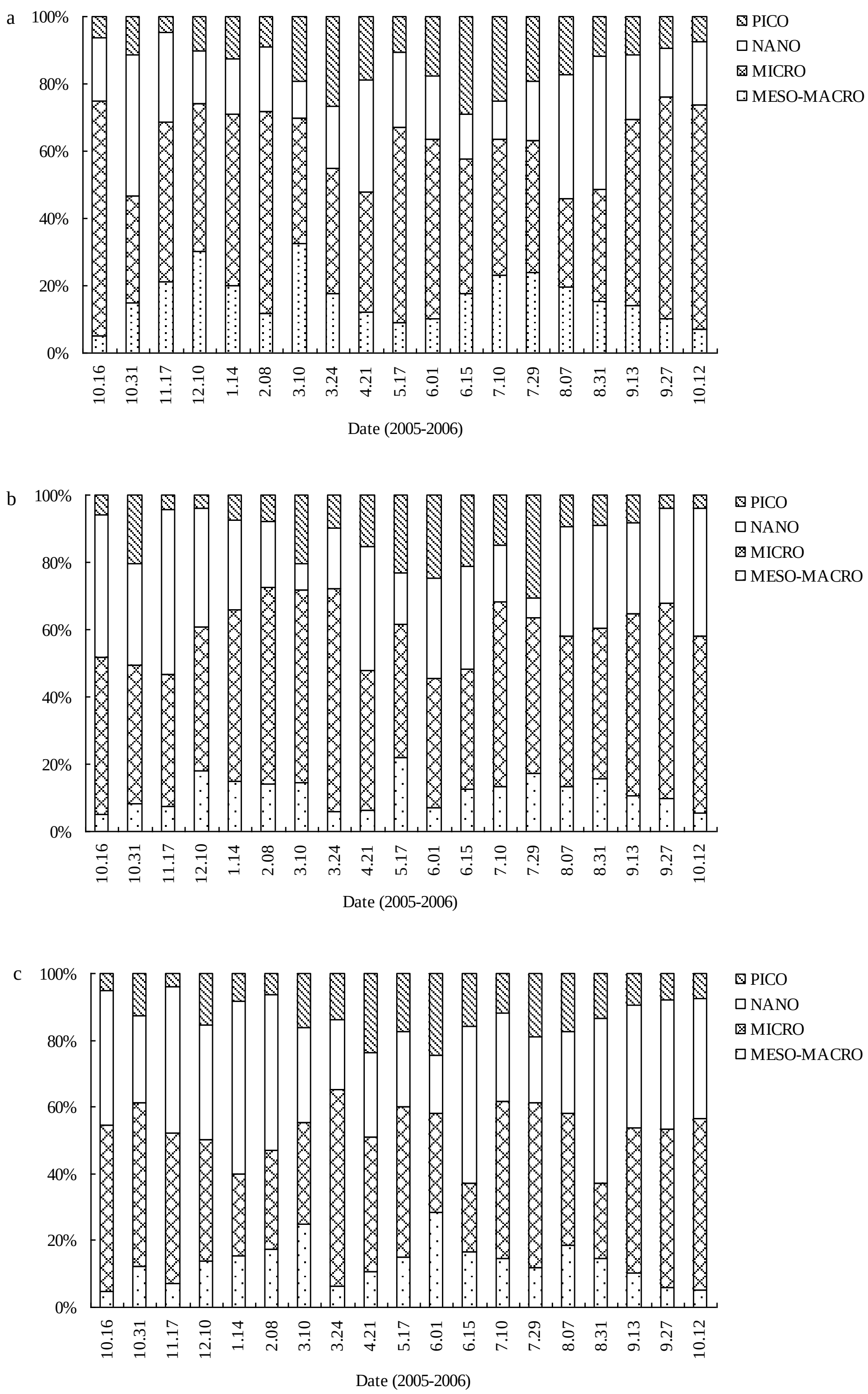

Figure 2. Annual variations of chlorophyll a percentages of size-fractionated phytoplankton in *Apostichopus japonicus* culture ponds (a: Number 3 pond, b: Number 4 pond, c: Number 9 pond).

pico) has greater competitive advantage in oligotrophic waters; Picophytoplankton usually predominates in sea areas with poor or intermittent nutrient supply; As eutrophication progresses and nutrients accumulate, microalgae with a short growth cycle dominate and the abundance of picoalgae declines (Stockner & Antia, 1986; Gobler, Cullison, Koch, Harder, & Krause, 2005; Totti *et al.*, 2005). Large phytoplankton (such as micros) has an advantage in temperate seas, and picophytoplankton plays an important role in tropical and subtropical seawaters (Takahashi & Bienfang, 1983; Bouteiller, Blanchot, & Podier, 1992). The investigated *Apostichopus japonicus* ponds were on the coast of the temperate North Yellow Sea of China in a eutrophic state, their phytoplankton community structure characteristics followed the basic rules given.

Table 2. Correlation coefficients (R) and *F* values between chlorophyll a and ecological factors in the monadic regressive analyses

Parameter	T	S	DO	pH	SD	NH_4^+-N	NO_3^--N	NO_2^--N	PO_4^{3-}-P	SiO_3^{2-}-Si
R	0.604	–0.627	–0.438	0.272	–0.51*	0.272	–0.504	0.533	0.411	0.705
F	9.78**	11.016*	3.793	1.360	6.25*	1.357	5.775*	6.737*	3.447	16.784**

The analyses had a critical probability level of 0.05 (*) or 0.01 (**).
T: water temperature, S: salinity, DO: dissolved oxygen, pH: pH value, SD: transparency.

Previous research indicated that the concentration and distribution of CHL-A in waters are mainly influenced by physical factors, nutrients, trace elements, organic pollutants and toxic substances (Calvo-Díaz *et al.*, 2008; Jiang *et al.*, 2010). In our study, regression analysis showed the similar results: the combined effects of various ecological factors (nutrients (SiO_3^{2-}-Si, NO_2^--N), salinity, water temperature, etc.) determined the CHL-A level in the *Apostichopus japonicus* pond. Especially, SiO_3^{2-}-Si concentration showed the most significant influence (the maximum PPC value) on CHL-A content indicating the importance of silicon in the ponds. Diatoms in particular have an absolute requirement for silicon, and they are by far the most important silicifying algal group in marine water and the dominating primary producers in the high-production areas of the ocean (Lewin, 1962; Kristiansen & Hoell, 2002). Our findings prompted the hypothesis that diatoms might well predominate in the phytoplankton community in *Apostichopus japonicus* culture pond that is beneficial to the cultured animals. Together with the result existed (microphytoplankton was always dominant in four seasons), we could deduce the micro-diatoms might be abundant in the investigated ponds at all seasons. However, the influences of some major ecological factors (water temperature and salinity) on microphytoplankton percentage were limited in the ponds. NO_2^--N was the second most important factor to CHL-A according to the PCC. Although its concentration peaked in summer with high temperature and low salinity and touched bottom in winter with high salinity and low temperature, there were no significant correlations among NO_2^--N, water temperature and salinity ($F < F_{0.05}$). During the study period, the NO_2^--N concentration was always at a low level that is safe for the cultured animals.

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