Distribution and seasonal variation of picoplankton in Sanggou Bay, China

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ABSTRACT: Picoplankton abundance and biomass in Sanggou Bay, China, were investigated in 4 successive seasons (April, August and October 2011, January 2012). Different distribution patterns of picoplankton abundance and biomass were observed according to season and culture areas (bivalves or macroalgae). Synechococcus, picoeukaryotes and heterotrophic prokaryotes exhibited higher abundance and biomass in warm seasons (summer and autumn) than in cold seasons (spring and winter). Over all 4 seasons, picoplankton abundance was higher in the bivalve culture area than in the macroalgae culture area. Among picoplankton, picoeukaryotes contributed most to the carbon standing stock in summer and autumn. In spring and winter, the heterotrophic component biomass exceeded that of the autotrophic picoplankton. Picoeukaryotes were an important contributor (21−27%) to total phytoplankton carbon biomass in spring to autumn. In spring, heterotrophic prokaryote biomass accounted for more than 56% of total phytoplankton biomass, and even exceeded phytoplankton biomass at some stations. As revealed by multiple stepwise regression analysis, physicochemical factors and protist grazing were the most important variables that controlled picoplankton distribution and variation. The reduction in grazing pressure, as well as phosphorus release by bivalves, is likely to explain the higher abundance of picoplankton in the bivalve culture area of Sanggou Bay.

KEY WORDS: Synechococcus · Picoeukaryotes · Heterotrophic prokaryotes · Sanggou Bay

INTRODUCTION

Marine picoplankton are generally defined as plankton in the size range ≤2 µm in diameter. Picoplankton consist mostly of cyanoprokaryotes of the genera Synechococcus (SYN) (Johnson & Sieburth 1979, Waterbury et al. 1979) and Prochlorococcus (Chisholm et al. 1988); picoeukaryotes (PEUK), a very diverse assemblage of eukaryotes; and heterotrophic prokaryotes (HP). Picoplankton have a ubiquitous distribution and contribute significantly to phytoplankton biomass and primary production in the ocean (Agawin et al. 2000, Bell & Kalff 2001). Picophytoplankton are major contributors to phytoplankton biomass in oligotrophic oceanic ecosystems (Li et al. 1983, Morán et al. 2004). SYN is present in inshore or coastal waters (Jochem 1988) and could account for 20% of the biomass of all living organ-
isms in the ocean (Caron et al. 1991). In some areas, PEUK are major biomass contributors (Worden et al. 2004). HP play a central role in the carbon flux in aquatic ecosystems. It is estimated that HP could consume ~50% of primary production and be responsible for 10–20% of daily organic matter production (Ducklow & Carlson 1992, Ducklow 2000).

The aquaculture of bivalves depends on the natural production of plankton. Suspension-feeding bivalves clear seston particles >3 µm in diameter from the water column (Newell 2004), and therefore adult bivalves cannot efficiently capture picoplankton. Although picoplankton do not directly contribute to the growth of bivalves, they can provide a large proportion of the food source for heterotrophic nanoflagellates and ciliates in the water column (Sherr & Sherr 2002). By quantifying the ingestion of protists feeding on picoplankton, it is possible to determine how bivalves can use the microbial energy indirectly (Le Gall et al. 1997). Furthermore, some bivalve larvae can use picoplankton as part of their food source (Gallager et al. 1994), and therefore the study of picoplankton abundance and biomass is considered to provide useful information on the microbial food web in aquacultural regions such as Sanggou Bay.

Sanggou Bay is a semi-circular bay on the northeastern coast of China, with a large entrance towards the Yellow Sea in the east. Sanggou Bay has been used for aquaculture for >20 yr (Guo et al. 1999). Nearly 2/3 of the area has been used for bivalves and seaweed aquaculture since 1983. The main cultivated species include the seaweed Laminaria japonica and longline culture of Chinese scallops Chlamys farreri and Pacific oysters Crassostrea gigas (Zhang et al. 2009). Although Sanggou Bay is one of the most important aquaculture areas for shellfish and seaweed in northern China, picoplankton distribution and seasonal variation, as well as their contribution to total phytoplankton biomass in Sanggou Bay, remain poorly documented. In the present study, we investigated picoplankton distribution over 4 successive seasons to gain insights into the factors and processes that regulate picoplankton abundance in Sanggou Bay.

MATERIALS AND METHODS

Study area and sampling strategy

Four cruises were conducted in Sanggou Bay (Fig. 1) over 4 successive seasons: April 2011 (spring), August 2011 (summer), October 2011 (autumn) and January 2012 (winter) using the fishing boat ‘Lu Rong Yu Yang 65536’. The bivalve culture areas (collectively referred to as ‘B-area’ here) are located at the head of the bay, and the macroalgae culture areas (‘M-area’) are located at the mouth of Sanggou Bay (Lu et al. 2015a). During each cruise, seawater samples were collected from the sea surface (0.5 m depth) at 19 stations (Fig. 1) using a Ruttner sampler (HYDROBIOS). In situ parameters such as water temperature and salinity were determined with a YSI® Professional Plus series multiprobe water quality meter.

Sample analysis

Seawater samples (5 cm³) for picoplankton flow cytometry analysis were fixed with paraformaldehyde (final concentration 1%) immediately after collection. After 15 min at room temperature, the samples were frozen in liquid nitrogen until analysis was carried out in the laboratory (Marie et al. 2000b).
Picoplankton flow cytometry analyses were run with a FACS Vantage SE flow cytometer (Becton Dickinson) equipped with a water-cooled Argon laser (488 nm, 1 W; Coherent). Protocols were adapted from the literature (Marie et al. 2000a,b). Fluorescent beads (2 µm; Polysciences; concentration unknown) were used as the internal standard for the instrument set-up and enumeration of picoplankton cells (Olson et al. 1993). For SYN and PEUK analysis, forward scatter, side scatter and 2 fluorescence signals (red, range: 695 ± 20 nm; orange, range: 585 ± 21 nm) were recorded. Signals were triggered on red fluorescence to discard signals from heterotrophic organisms and inorganic particles. SYN and PEUK were distinguished on the basis of their scatter and fluorescence signals.

For HP analysis, seawater sub-samples were diluted 5-fold with TE buffer (Tris-EDTA, 100 mM Tris-Cl, mM EDTA, pH 8.0; Sigma), and then stained with the nucleic acid dye SYBR Green I (Molecular Probes; final dilution 10⁻⁴, v/v) and kept in the dark at room temperature for 20 min before analysis. HP cell groups were resolved on the basis of their green (range: 530 ± 15 nm) fluorescence signal in the green fluorescence vs. sideward scatter cytogram.

For the determination of chlorophyll (chl) a concentration, 50–200 cm⁻³ seawater samples were filtered onto GF/F glass-fibre filters (Whatman) under low vacuum. The filters were wrapped in aluminium foil and kept frozen at −80°C until analysis in the laboratory. Chl a was extracted with 90% acetone at 4°C in the dark for 20 h. Chl a concentrations were determined by the acidification method using a Turner Design (Model Trilogy 040) fluorometer, which was calibrated with pure chl a (Sigma) (Parsons et al. 1984).

Seawater samples for determining nutrient concentration were filtered through acid-washed, pre-cleaned (with ultrapure water), 0.45 µm pore-size acetate cellulose filters (Development Center of Water Treatment Technology, Hangzhou, PR China). The filtrates were poisoned by the addition of saturated HgCl₂ (ca 1.5 × 10⁻³ v/v), preserved in low-density polyethylene bottles at room temperature and then analysed in the laboratory.

Nutrient concentrations including those of NO₃⁻ and NO₂⁻ were determined spectrophotometrically using a SKALAR SAN plus autoanalyser, while NH₄⁺ and PO₄³⁻ concentrations were determined by manual methods (Parsons et al. 1984). The concentration of dissolved inorganic nitrogen (DIN) was calculated as the sum of NO₃⁻, NO₂⁻ and NH₄⁺.

The enumeration of heterotrophic nanoflagellates (HNF) followed specifications by Lu et al. (2015a). The enumeration of ciliates was carried out according to Yu et al. (2013). Picoplankton biomass was derived from the abundance of the cell groups resolved by flow cytometry. The abundance/biomass conversion factors used for SYN, PEUK and HP were 250 fg C cell⁻¹ (Li et al. 1992), 1500 fg C cell⁻¹ (Zubkov et al. 1998) and 20 fg C cell⁻¹ (Lee & Fuhrman 1987), respectively. Total phytoplankton biomass per unit volume was estimated from the chl a concentration assuming a constant C:chl a ratio of 50 (mg:mg) (Krempin & Sullivan 1981).

Flow cytometry data were collected and analysed with CellQuest software (version 3.3, Becton Dickinson). Contour plots were generated using Surfer (version 8.0, Golden Software). Statistical analysis was conducted using SPSS (version 19, IBM SPSS Statistics). Two independent-sample t-tests were used to compare picoplankton abundance between the B- and M-areas. Spearman correlation analysis was used to detect significant relationships between variables. As an attempt to explain the variation in picoplankton distribution, stepwise multiple regression analysis was performed to assess the relative influence of potential factors controlling picoplankton abundance (temperature, salinity, nutrient and chl a concentrations and other biological components). The abundance data of picoplankton, HNF and ciliates used for statistical analysis were log-transformed to achieve homogeneity of the variance.

RESULTS

Physicochemical conditions

The seasonal distribution of seawater variables is shown in Fig. 2. The average surface water temperature of Sanggou Bay was 9.00, 21.36, 16.47 and 3.76°C in spring, summer, autumn and winter, respectively (Table 1). In spring and summer, water temperature decreased from inside Sanggou Bay to the open sea, while the opposite trend was observed in autumn and winter. Salinity increased from inside the bay to the open sea in summer, autumn and winter. Minimum average salinity was found in summer. In summer and autumn, high chl a concentrations were observed, especially at coastal stations inside the bay. Maximum chl a concentration reached 38.74 mg m⁻³ at Stn SG13 in summer. Average DIN varied from 4.83 µM in summer to 10.44 µM in autumn. The season-averaged PO₄³⁻ concentration was much lower than that of DIN with a maximum (0.11 µM) in spring and a minimum (0.02 µM) in autumn and winter. At some stations in autumn and winter, PO₄³⁻ concentration was below the detection limit (Fig. 2).
Depending on seasons, different distribution patterns of picoplankton abundances were observed in Sanggou Bay (Fig. 3). In spring, SYN abundance was lower at the centre of the bay than at other stations. In summer and autumn, SYN abundance decreased from coastal stations inside the bay to the open sea; however, the opposite trend was observed in winter. Average SYN abundance varied from $0.05 \times 10^3$ cells cm$^{-3}$ in spring to $84.06 \times 10^3$ cells cm$^{-3}$ in autumn, with a difference of about 4 orders of magnitude (Table 1). The season-averaged SYN abundance was significantly higher in summer and autumn than in spring and winter ($p < 0.01$).

PEUK and HP had similar abundance distribution patterns in Sanggou Bay, with seasonal variation in the order winter < spring < autumn < summer. Over all seasons, PEUK and HP abundances decreased from coastal stations inside the bay to the open sea. Both PEUK and HP abundances fluctuated less than that of SYN, with values from $1.80 \times 10^3$ cells cm$^{-3}$ and $3.00 \times 10^5$ cells cm$^{-3}$ in winter to $82.57 \times 10^3$ cells cm$^{-3}$ and $40.77 \times 10^5$ cells cm$^{-3}$ in summer, respectively (Table 1). The abundances of PEUK and HP were significantly higher in summer than in other seasons ($p < 0.01$).
The relationships between picoplankton abundance and environmental and biological factors were complex. In spring, PEUK and HP abundances were positively correlated with each other (Table 2). Both PEUK and HP abundances were positively correlated with water temperature, salinity and ciliate abundance. No significant correlation was found between SYN abundance and the other parameters. In summer, SYN abundance was positively correlated with PEUK and negatively correlated with salinity. HP abundance was positively correlated with HNF abundance, temperature and DIN, and negatively correlated with salinity. The abundances of all 3 picoplankton groups were positively correlated with chl a concentration. In autumn, SYN, PEUK and HP abundances were positively correlated with each other, as well as with HNF and ciliate abundances, and chl a and PO$_4^{3-}$ concentrations, while they were negatively correlated with temperature and salinity. In winter, no significant correlation was found between picoplankton groups. SYN abundance was positively correlated with temperature and chl a and negatively correlated with HNF and ciliate abundances. PEUK abundance was positively correlated with chl a concentration. HP abundance was negatively correlated with chl a concentration.

### Distribution of picoplankton in different aquaculture areas

In warm seasons (summer and autumn), there was an obvious freshwater input to the bay (Fig. 2). All picoplankton groups exhibited higher abundances in the B-area than in the M-area, especially in autumn (Fig. 3, Table 1). In cold seasons (winter and spring), SYN abundance remained low throughout Sanggou Bay, with slightly higher values in the M-area (Fig. 3, Table 1). For PEUK and HP, the abundances were still higher in the B-area, but the difference was not significant in winter.

As revealed by multiple stepwise regression analysis, warm-season grazing by protists was the most significant.
important variable that controlled picoplankton abundance and distribution in the M-area (Table 3). HNF abundance explained 66.5 and 80.8% of the variance for SYN and PEUK, respectively. For HP, chl $a$ was the most important variable; however, HNF and ciliate abundance also explained about 8.3% of the abundance and distribution of HP. In the B-area, during warm seasons, physicochemical factors and HNF provided the best explanation for SYN and HP distribution, respectively. No significant variable was found for PEUK. In cold seasons, salinity was the most important variable controlling the distribution of picoplankton in both M- and B-areas (Table 3).
Table 3. Summary of multiple stepwise regression analysis between picoplankton abundances and environmental and biological variables in culture areas of Sanggou Bay and adjacent area (M-area: macroalgae culture, B-area: bivalve culture; see Fig. 1) in warm (summer, autumn) and cold (winter, spring) seasons. Picoplankton, heterotrophic nanoflagellate (HNF) and ciliate abundances were log transformed prior to analysis. $R^2$: correlation coefficient of multiple determination. $R^2$ change: change in multiple $R^2$ caused by entering a new variable in a single step (hierarchical analysis). Results of $F > 1$ and $p < 0.05$ represent improvements due to fitting the regression model is much greater than the inaccuracy within the model, which means the final model significantly improved our ability to predict the outcome variable. NS: not significant. SYN: *Synechococcus*; PEUK: picoeukaryotes; HP: heterotrophic prokaryotes; Chl: chl $a$ concentration; T: temperature; S: salinity

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Fig. 4. Depth-averaged biomass (mg C m$^{-3}$) of picoplankton in Sanggou Bay over 4 successive seasons. SYN: *Synechococcus*; PEUK: picoeukaryotes; HP: heterotrophic prokaryotes. Note different y-axis scales

**Carbon biomass contribution of picoplankton to phytoplankton**

Among picoplankton, PEUK represented the highest standing stock of carbon biomass in summer and autumn (Fig. 4), contributing to >50% of picoplankton biomass (Fig. 5). In spring and winter, the heterotrophic component of biomass exceeded that of autotrophic picoplankton. HP was the major contributor to picoplankton biomass in spring and winter. SYN biomass was relatively low compared with that of PEUK and HP throughout the 4 successive seasons (Fig. 4).
In spring, summer and autumn, PEUK was an important (21.46−27.74%) carbon contributor to total phytoplankton biomass (Fig. 6). This contribution decreased to 6.39% in winter. HP biomass amounted to >50% of total phytoplankton biomass in spring, and at some stations even exceeded phytoplankton biomass. SYN contributed 6.82% to phytoplankton biomass in autumn and <1.5% in other seasons.

Fig. 5. Biomass contribution of *Synechococcus* (SYN), pico-eukaryotes (PEUK) and heterotrophic prokaryotes (HP) to picoplankton biomass in Sanggou Bay over 4 successive seasons

Fig. 6. Biomass contribution of *Synechococcus* (SYN), picoeukaryotes (PEUK) and heterotrophic prokaryotes (HP) to total phytoplankton biomass in Sanggou Bay over 4 successive seasons. The total phytoplankton biomass was derived from chlorophyll a concentration. Note different y-axis scales

**DISCUSSION**

**Picoplankton seasonal distribution and variation**

This is the first report on picoplankton abundance distribution and its seasonal variation in Sanggou Bay, China, with results comparable to those reported from other coastal waters (Vaquer et al. 1996, Kamiyama 2004, Bec et al. 2005, Kamiyama et al. 2009, Thomas et al. 2010, Bouvy et al. 2012). Picoplankton abundance distribution and its variations depend on both abiotic and biotic factors. Abiotic factors, also called bottom-up controls, include water temperature and salinity, as well as light and nutrient availability. The biotic factors (top-down controls) are essentially predation by nano- and micro-zooplankton, and lysis by virioplankton.

Picoplankton abundance is particularly affected by water temperature and nutrient availability (Agawin et al. 2000). Seasonal variation of SYN and HP abundances in temperate waters usually follows patterns with maxima in summer and minima in winter (Li 1998). In Sanggou Bay, a clear seasonality for picoplankton abundance and biomass was observed, associated with physicochemical features. High abundances and biomasses of SYN, PEUK and HP were found during summer and autumn, in agreement with previous reports (Vaquer et al. 1996, DuRand et al. 2001, Bec et al. 2005). SYN abundance is about 4 orders of magnitude higher in
autumn than in spring in Sanggou Bay. Similar SYN variation was also found in previous studies (Durand et al. 2001, Li & Dickie 2001, Agawin et al. 2003). A 10 yr monthly observation (2006–2015) in Jiaozhou Bay, China, at a similar longitude and latitude, has revealed the same variation (T. Xiao, L. Zhao unpubl. data).

**Picoplankton in the aquaculture area**

Being an important component of the aquatic food web, picoplankton feed larger zooplankton that channel their carbon biomass from microbial to higher trophic levels (Azam et al. 1983). The aquaculture of bivalves depends on the production of natural plankton. Picoplankton (<2 µm) are too small to be efficiently retained by most bivalves, including the scallop *Chlamys farreri* and oyster *Crassostrea gigas* (Barillé et al. 1993, Kreeger & Newell 1996, Hawkins et al. 2001). However, the distribution of picoplankton is still affected by bivalves in aquaculture areas. Although picoplankton do not directly contribute to the growth of bivalves, they can provide a large proportion of the food source for HNF and ciliates in the water column (Sherr & Sherr 2002). Most HNF (2–20 µm) and ciliates (mostly >10 µm) are much larger and can be efficiently captured by most bivalves (Riisgård 1988, Fournier et al. 2012). Therefore, bivalves can use the microbial energy indirectly (Le Gall et al. 1997). Nano- and micro-zooplankton grazing are important top-down control factors for picoplankton (Sherr & Sherr 2002). Protists are recognized as the main consumers of SYN, PEUK and HP in similar environments (Kamiyama 2004, Bec et al. 2005). In the macroalgae culture area of Sanggou Bay, grazing by protists was the most important variable controlling picoplankton abundances and distribution in both warm and cold seasons. However, no such correlation could be observed in the bivalve culture area. Physicochemical factors such as temperature and salinity were the main control factors of picoplankton distribution. It is possible that HNF and ciliates in the B-area were efficiently retained by bivalves, alleviating grazing pressure on picoplankton and enabling a significantly higher abundance of picoplankton in this area, especially in warm seasons. We found possible collinearity between some variables used in the stepwise regressions (Table 3). It is possible that the estimates of the multiple regressions may change erratically in response to small changes in the data. To remedy the analysis, data were log transformed prior to analysis to standardize the variables in our study. Despite its shortcomings, stepwise regression has nevertheless been a suitable method used to predict influential factors in other research (e.g. Kimmel et al. 2012, Oberbeckmann et al. 2012).

A predominance of picoplankton has also been reported in other areas of intense bivalve farming (Dupuy et al. 2000). Traditionally, picophytoplankton has been viewed as having a critical growth dependence on inorganic nutrients. At low nutrient concentrations, picoplankton cells can take up nutrients better than large plankton cells, owing to their higher surface area to volume ratio (Morel et al. 1991, Chisholm 1992). In Sanggou Bay, phosphorus (P) was found to be deficient, whereas DIN was sufficient (Sun et al. 2007). Bivalve culture can release P into the environment (Carlsson et al. 2012, Cranford et al. 2012), and the release of P by bivalves may have induced the high abundance of picoplankton in the Sanggou Bay B-area. Indeed, a 7 d *in situ* enclosure experiment in Sanggou Bay demonstrated that scallop cultivation increased the PO$_4^{3-}$ concentration, as well as the abundance of picoplankton, total nanoflagellates and ciliates (Lu et al. 2015a,b). In addition to P release, bivalves can excrete important amounts of ammonium ions, which also favours picophytoplankton (Chisholm 1992, Courties et al. 1994). Although we lack data on ammonium ion concentration in Sanggou Bay, it is possible that ammonium ions stimulated the growth of picoplankton in the bivalve culture area.

**Picoplankton biomass contribution**

In cold seasons (winter and spring), heterotrophic picoplankton carbon biomass exceeded that of autotrophic picoplankton. This result is in agreement with observations in the Sargasso Sea, where the microbial carbon biomass was dominated by non-photosynthetic prokaryotes (Fuhrman et al. 1989, Bouvy et al. 2012). When the water temperature rose, PEUK biomass became predominant (>50%) within the picoplankton biomass. These results differ from previous observations in distinct environments such as the northeastern Atlantic Ocean (Partensky et al. 1996), the South Pacific Ocean (Grob et al. 2007) and the Yellow Sea (Zhao et al. 2011), where SYN or HP was predominant in the picoplankton biomass. Bec et al. (2005) reported that PEUK were predominant within picoplankton, and could serve as an important carbon source for the protozoan community. Our observations are in line with these findings, support-
ing the suggestion that PEUK could make a large contribution to the carbon flow towards higher trophic levels in coastal regions.

CONCLUSION

Our study is the first report on picoplankton seasonal abundance distribution and its variations in Sanggou Bay, China. Different distribution patterns of picoplankton abundance and biomass were observed. Physicochemical factors and protist grazing were the most important variables controlling the distribution of and variation in picoplankton abundance in Sanggou Bay. Picoplankton were more abundant in the bivalve culture area than in the macroalgae culture area, especially in warm seasons. Among the picoplankton, PEUK contributed most to the carbon biomass standing stock in summer and autumn. The reduction in protist grazing pressure, as well as P release by bivalves, are likely explanations for the higher picoplankton abundance in the bivalve culture area. In spring and winter, the heterotrophic component of the biomass exceeded that of the autotrophic picoplankton. In spring to autumn, PEUK contributed >20% to the assessed autotrophic biomass. HP biomass amounted to >56% of the assessed autotrophic biomass in spring, and at some stations the percentage was even larger.

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LITERATURE CITED


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