

# Size fraction of phytoplankton and the contribution of natural plankton to the carbon source of Zhikong scallop *Chlamys farreri* in mariculture ecosystem of the Sanggou Bay

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## Abstract

The biomass and size fraction of phytoplankton in terms of chlorophyll *a* (Chl *a*) was measured during four cruises conducted in April, July, October 2013 and January 2014 in mariculture area, the Sanggou Bay, China. Results show that total Chl *a* levels in the surface seawater of the Sanggou Bay generally range from 0.10 to 20.46 µg/L, with an average value of 2.13 µg/L. Nano-phytoplankton was the most important size-fraction and accounted for about 65.1% of total Chl *a*. In order to evaluate the importance of the “protozoan trophic link” for energy transfer from the microbial loop to filter-feeding feeders, Zhikong scallop *Chlamys farreri* was then offered a natural planktonic community as potential prey. Results show that scallops obtained carbon source from natural plankton with the rate of 11 033.05 µg/(g·d). Protists (nanoflagellates and ciliates) were the dominant source of carbon retained by scallop (48.78%). The microbial loop provided 58.45% of the carbon source for farmed scallops. These results indicate that the microbial loop represent a valuable trophic resource in mariculture system of the Sanggou Bay.

**Key words:** phytoplankton, picoplankton, protist, microbial food web, *Chlamys farreri*

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## 1 Introduction

China is the largest mariculture country in the world and produces 17.39 million tonnes of seafood in 2013, worth approximately 260.45 billion RMBs (Fisheries Bureau of Ministry of Agriculture, China, 2014). Among the total mariculture production, the production of shellfish takes about 73.18% at an amount of 12.73 million tonnes. Filter-feeding bivalves, such as oysters, scallops, mussels, etc., are the main species and occupied about 90.37% of the total shellfish harvest. Bivalves obtain energy resources by filtering particles from seawater and their growth depends on the nutritive value of the retained seston. The seston consists of plankton of a wide range of sizes and palatability, material re-suspended from the benthos, as well as aggregates consisting of high molecular weight substances, detritus, fecal pellets and microorganisms (Crocker and Passow, 1995; Passow et al., 1994). Stable isotope studies, like morphological-based gut content analyses, have demonstrated the primary importance of phytoplankton (Yokoyama et al., 2005; Marín Leal et al., 2008) and microphytobenthic material (Sauriau and Kang, 2000; Kang

et al., 2006) in the diets of suspension feeding bivalves. Phytoplankton can be divided into three size classes: micro-phytoplankton (>20 µm), nano-phytoplankton (2.0–20 µm) and picophytoplankton (0.45–2.0 µm) and different size-fractionated phytoplankton carry out different ecological functions (Huang et al., 1999; Kormas et al., 2002; Froneman et al., 2004). However, the gill of the bivalves, which is a dominant feeding organism, cannot retain pico-phytoplankton effectively (Kreeger and Newell, 1996; Yukihiro et al., 1999). Heterotrophic protists are an important component of nanoplankton and microplankton assemblages in the marine pelagic ecosystem (Sherr and Sherr, 1994). With their small size and high growth rates, ciliates and heterotrophic flagellates may contribute significantly to trophic flux and nutrient cycling (Sheldon et al., 1986). Heterotrophic protists consume bacteria and phytoplankton and are themselves important prey items for mesozooplankton (Karl et al., 2003). The linked concepts of “microbial loop” and “protozoan trophic link” have been very well documented in filter-feeding microzooplankton such as copepods, but have not been applied

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to energy transfer to filter-feeding bivalves, with the exception of the recent demonstration of ciliates and flagellates assimilation by oyster and mussels (Le Gall et al., 1997; Dupuy et al., 1999, 2000; Loret et al., 2000; Wong et al., 2003). This raises the question of the role of heterotrophic protists as a valuable trophic link between picoplankton and filter-feeding bivalves under cultivation.

The Sanggou Bay, located in Shandong Province of China, is one of the most intensive mariculture bays in China. It has been under mariculture for more than 30 years. The main cultivation species were seaweed *Saccharina japonica*, oyster *Crassostrea gigas* and scallop *Chlamys farreri* with the annual production 84 500 tons (DW), 60 000 tons (WW) and 15 000 tons (WW) respectively (Rongcheng Fisheries Technology Extension Station, 2012, www.rchy.gov.cn). As for the main food source of the bivalves, a number of studies on phytoplankton communities including their spatial and temporal variations have been conducted in Sanggou Bay (Song et al., 2007; Mu et al., 2009; Li et al., 2010; Hao et al., 2012), but mostly for the entire phytoplankton community rather than for specific size-fractions. To fill this gap, this study aims to describe seasonal variations of Chl *a* biomass and the relative contribution of different size classes to total Chl *a* in the Sanggou Bay by means of size fractionation in the first step, and then to assess the contribution of the autotrophic and/or heterotrophic plankton (heterotrophic bacteria, *Synechococcus*, picoeukaryotes, nanoflagellates, ciliates) to the carbon source of scallop in autumn by flow-through chamber method.

## 2 Materials and methods

### 2.1 Description of the study area

The study was carried out at the aquaculture area of the Sanggou Bay (37°01'–37°09'N, 122°24'–122°35'E), Shandong Province, China. The bay is a small semi-enclosed bay (about 144 km<sup>2</sup>) with a mean depth of 7.5 m. Water exchange between the bay and Yellow Sea is driven by a semi-diurnal tide (tidal range 2 m) through an 11.5-km opening area of the bay (Jiang et al., 2007). Rivers which flow into the Sanggou Bay include the Gu River, Sanggan River, and Shili River, with the annual water discharge  $(1.7\text{--}2.3)\times 10^8\text{ m}^3$  (Annual Report on the River Regime of Rongcheng City 2011, www.rcsl.gov.cn). Among them, Gu River is the largest River and occupies almost 70% of the total water

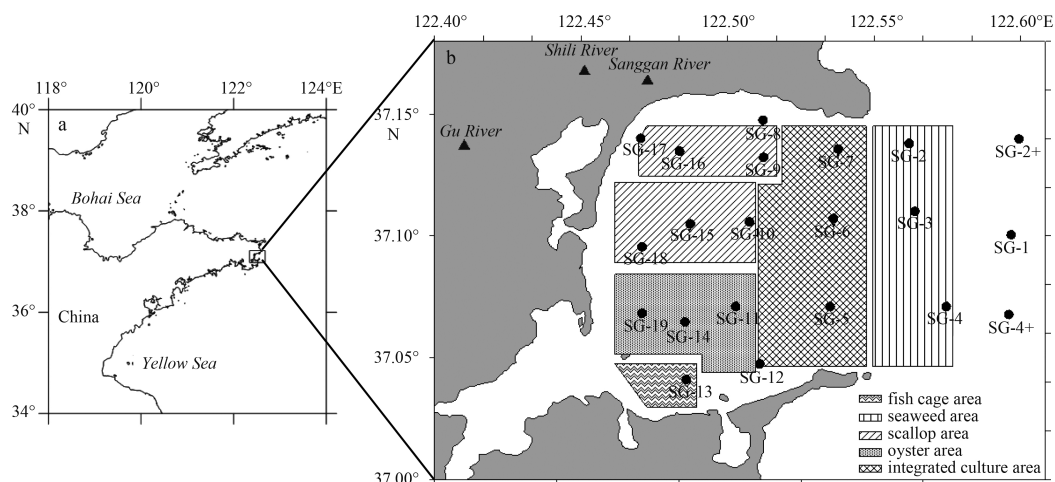
discharge. The Zhikong scallop *Chlamys farreri* is one of the main species cultured and it is mainly grown in lantern nets suspended underneath buoyed longlines. A lantern net is generally divided into 8–10 tiers by plastic perforated plates, with stocking density ca. 350 individuals per lantern net. Growout period of the cultured Zhikong scallop is from 1.5 to 2 years.

### 2.2 Experimental design, sample collection and analysis procedures

Four survey cruises were performed in the Sanggou Bay at the 21 stations during April 22 to 25, July 21 to 25, October 17 to 20, 2013 and January 15 to 17, 2014 (Fig. 1). Surface seawater samples (0.5 m below the sea surface) were collected by acid-cleaned 5 L Hydro-bios water sampler and stored in polyethylene bottles. Duplicate subsamples of 1 000 mL seawater samples were sequentially filtered at room temperature after collection onto meshes of 20  $\mu\text{m}$  and polycarbonate 47 mm (Millipore) filters of 2  $\mu\text{m}$  and 0.45  $\mu\text{m}$  of pore size in turn to separate three size classes: micro-phytoplankton (>20  $\mu\text{m}$ ), nano-phytoplankton (2.0–20  $\mu\text{m}$ ) and pico-phytoplankton (<2.0  $\mu\text{m}$ ). The filters were then wrapped in foil and frozen at –20°C before extraction, generally within one week. Phytoplankton cells retained on each size-fractionated mesh or filter were extracted with 90% acetone at 4°C and stored in the dark for 24 h. The contents were subsequently centrifuged for 15 min, after which the clear supernatant fluid was analyzed with a Trilogy Fluorometer (Turner Designs, USA). Chl *a* concentration was calculated according to method given by Parson et al. (1984). Dissolved inorganic nutrients (nitrates, nitrites, ammonium and phosphate) analyses were carried on filtered water (0.45  $\mu\text{m}$ ) spectrophotometrically according to methods described by Strickland and Parsons (1972).

In addition, seawater transparency was measured by plastic secchi disc. Sea surface temperature (SST), salinity and pH were measured using an YSI Professional Plus handheld multi-parameter water quality meter (Yellow Springs Instrument Company, USA). The daily primary production was estimated by the Chlorophyll method (Jiang et al., 2012). Daily illumination time for each sampling day was obtained from website <http://www.weather.com.cn/weather/101121304.shtml>. Data on percentage of phytoplankton extracellular release (PER) was obtained from published literature (Liu, 2012).

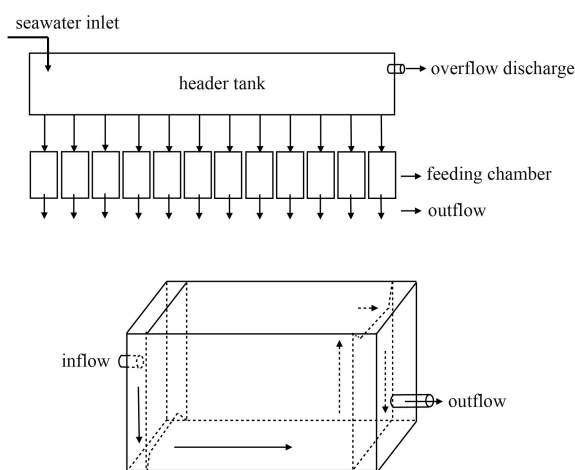
Scallops *Chlamys farreri* were collected from the commercial



**Fig. 1.** Map of field observation locations in the Sanggou Bay, Shandong Province, China, during the survey cruises from April 2013 to January 2014.

suspended lantern nets farm in the Sanggou Bay during October 2014. Scallops ( $n=45$ ) selected were  $(18.17\pm 3.77)$  g wet weight and  $(55.51\pm 1.77)$  mm shell height. The valves were cleaned before transport to the acclimation system, where they were acclimated for 2 d in flow-through seawater aquaria with a capacity of 200 L at ambient temperature and salinity ( $(17.4\pm 0.6)^\circ\text{C}$  and  $31.05\pm 0.03$ ) prior to the start of the experiment.

The feeding experiments were performed by transferring scallops from the acclimatization tank to individual flow-through chambers (Fig. 2a). The chambers were continuously supplied with flowing unfiltered seawater from header tank. The internal dimensions of those chambers were 23.6 cm (width) $\times$ 48.8 cm (length) $\times$ 28.7 cm (height) and received a continuous flow of seawater (Fig. 2b). This chamber design restrains internal recirculation and helps to prevent refiltration of the water by the scallops (Strohmeier et al., 2009). In this experiment, twelve chambers were used, of which nine were filled with one individual and three served as a control without scallop. Flow rates through each chamber were adjusted to 200–400 mL/min and determined by simultaneously collecting and measuring the volume of water collected from each outflow. The scallops were left undisturbed in chambers with flowing seawater at least 0.5 h to resume feeding before sampling water from the outlet of the chambers. The scallops was exposed to natural photoperiod (11L: 13D) throughout this study. Temperature, salinity and pH were simultaneously measured at 10 min intervals in the header tank using an YSI Professional Plus handheld multi-parameter water quality meter (Yellow Springs Instrument Company, USA).



**Fig. 2.** Schematic diagram of the flow-through experiment system (a) and feeding chamber (b).

Water samples (1 000 mL) collected from each chamber outlet were filtered on Millipore filters of 2  $\mu\text{m}$  and 0.45  $\mu\text{m}$  of pore size in turn to separate size classes. Phytoplankton cells retained on each size-fractionated mesh or filter were extracted with 90% acetone at  $4^\circ\text{C}$  and stored in the dark for 24 h. The contents were subsequently centrifuged for 15 min, after which the clear supernatant fluid was analyzed with a Trilogy Fluorometer (Turner Designs, USA). Chl  $a$  concentration was calculated according to method given by Parson et al. (1984). To convert Chl  $a > 2 \mu\text{m}$  and Chl  $a < 2 \mu\text{m}$  concentrations into carbon biomass, we used ratios equal to 50  $\mu\text{g}/\mu\text{g}$  (C/Chl  $a$ ) and 82  $\mu\text{g}/\mu\text{g}$  (C/Chl  $a$ ), respectively (Charpy and Charpy-Roubaud, 1990; Charpy, 1996).

Water samples (200 mL) collected from each chamber outlet

were analyzed for the abundances of picoplankton (cell/mL), nanoflagellates (ind./mL) and ciliates (ind./L). In this study, picoplankton abundance is defined as the sum of heterotrophic bacteria, *Synechococcus* and picoeukaryotes abundances. The abundances of picoplankton were counted by flow cytometry using a FACS vantage SE system (Becton Dickinson) equipped with a water-cooled Argon laser (488 nm, 1 W, Coherent) (Zhao et al., 2011). To calculate an average carbon conversion factor for picoplankton, we used conversion factors of 14 fg/cell (Gundersen et al., 2002), 178 fg/cell (Charpy and Blanchot, 1998) and 836 fg/cell (Verity et al., 1992) for bacteria, *Synechococcus* and picoeukaryotes, respectively.

Samples (100 mL) used to estimate the abundance of nanoflagellates were pre-filtered through a nylon mesh (20  $\mu\text{m}$  pore size), then fixed immediately with glutaraldehyde at a final concentration of 0.5% (v/v). Subsequently, subsamples (10–20 mL) were filtered in triplicate onto a polycarbonate black membrane (0.22  $\mu\text{m}$ ) (Millipore) at low pressure (<100 mm Hg). Cells left on the filter membranes were stained immediately by adding 4', 6-diamidino-2-phenylindole (DAPI) at a concentration of 10  $\mu\text{g}/\text{mL}$  for 15 min and examined with an epifluorescence microscope (Leica DM 4500B) at 1 000 $\times$  magnification. The sizes of the cells were measured using Leica DM4500 software. To obtain a reliable estimation of the abundance, at least 100 cells were counted per sample (Lu et al., 2015). The carbon-biovolume conversion factor of 220  $\text{fg}/\mu\text{m}^3$  was used for nanoflagellates (Lu, 2014).

To determine the total abundance of ciliates, 1 000 mL of seawater was fixed with acid Lugol's iodine solution (at a final concentration of 1%) and stored at  $4^\circ\text{C}$  in the dark. After settling for 48 h, the supernatant was siphoned out until 150 mL of sample remained. The concentrated sample was settled in a Utermöhl chamber and then counted using an inverted microscope at 200 $\times$  or 400 $\times$ . Ciliates were counted, and the length and diameter of each cell were measured. The carbon-biovolume conversion factor for ciliates was 0.19  $\text{pg}/\mu\text{m}^3$  (Putt and Stoecker, 1989).

The individual clearance rates ( $CR$ , L/(ind. $\cdot$ h)) for the components of the microbial food web were calculated according to the following equations (Hildreth and Crisp, 1976):

$$CR = [(C_1 - C_0) / C_1] \times F / 100,$$

where  $C_1$  and  $C_0$  are the abundance counts in the control and experimental chamber respectively.  $F$  is the flow rate (L/h) measured at the outlet of each chamber.

At the end of the experiments, each scallop height was measured and the flesh was freeze dried and weighted. Freeze dried fresh weight (DW in g) was used to normalize clearance rates per gram of dry flesh ( $CR_w$ , L/(g $\cdot$ h)). Measurements were done repeatedly (2–3 times) at 4 h time interval. The experiment was completed after 12 h exposure to ambient seawater.

To assess the contribution of each plankton type to the diet of *Chlamys farreri*, we estimated carbon retention rates by the following equation (Fournier et al., 2012):

$$RR = CB \times CR_w,$$

where  $RR$  is retention rates of carbon in  $\mu\text{g}/(\text{g}\cdot\text{h})$ ,  $CB$  is carbon biomass in  $\mu\text{g}/\text{L}$ .

### 2.3 Statistics analysis and contour plots

Statistics were done using the software SPSS 17.0 for Windows. The one-way analysis of variance (ANOVA) was used to

analyze the effects of season on variations in Chl *a* concentration. According to the results of homogeneity test, a posteriori, Tukey's HSD or Tamhane's T2 comparisons were applied to determine statistically significant differences ( $p < 0.05$ ) following ANOVA. Contour maps were plotted by Surfer 8.0. A CCA (Canonical Correspondence Analysis) was used to evaluate the combined main environmental variables and the fractioned phytoplankton. A Monte Carlo Test proved that the correlation among the biological and abiotic matrices was statistically significant ( $p = 0.01$ ).

### 3 Results

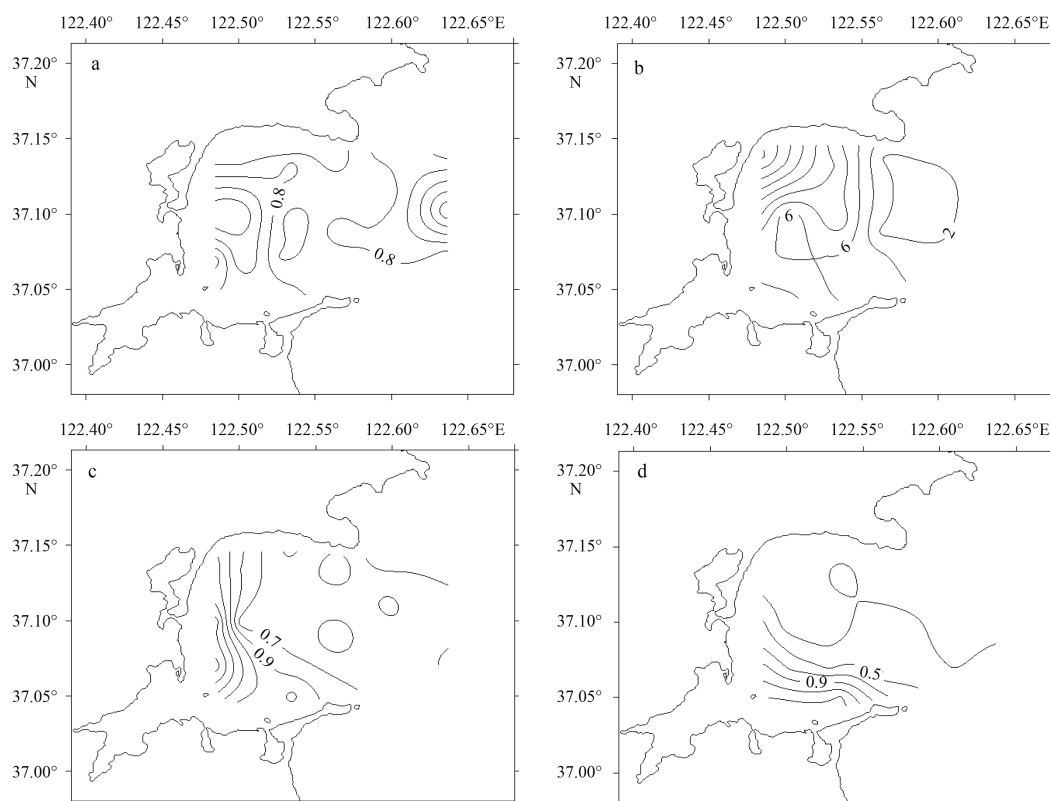
#### 3.1 Spatial, seasonal and size-fraction variations of Chl *a* concentration

The spatial distributions of Chl *a* concentration in different seasons are depicted in Fig. 3. During spring, the surface water of the bay had a homogeneous Chl *a* concentration of  $(0.83 \pm 0.45)$   $\mu\text{g/L}$  (Fig. 3a). In summer, the highest values were found along the northwestern coast of the bay with the maximum of 20.46

$\mu\text{g/L}$  (Fig. 3b). The Chl *a* concentration inside the bay was much higher than outside area. In autumn, the Chl *a* concentration showed a clear increasing trend from outside to inside the bay. The higher Chl *a* concentration was along the west coast of the bay with the values ranged between 1.14 and 2.00  $\mu\text{g/L}$ . Lowest concentrations were found in the mouth area of the bay, with concentrations as low as 0.36  $\mu\text{g/L}$  (Fig. 3c). In winter, the highest values were found along the southwestern coast of the bay with the maximum of 1.66  $\mu\text{g/L}$  (Fig. 3d).

Total Chl *a* concentration and primary production values in the four cruises are displayed in Table 1. Median concentrations of Chl *a* varied significantly among sampling periods ( $p < 0.01$ ) with the highest values observed in summer and the minimum in winter. Intermediate values of Chl *a* concentrations were observed in spring and autumn. Mean transparency ranged from  $(0.86 \pm 0.27)$  m (in April 2013) to  $(2.23 \pm 1.22)$  m (in January 2014).

Seasonal variation of primary production shows the same trend as Chl *a*. The mean annual primary production in the Sanggou Bay area was 180.32  $\text{mg}/(\text{m}^2 \cdot \text{d})$ . One-way ANOVA indic-



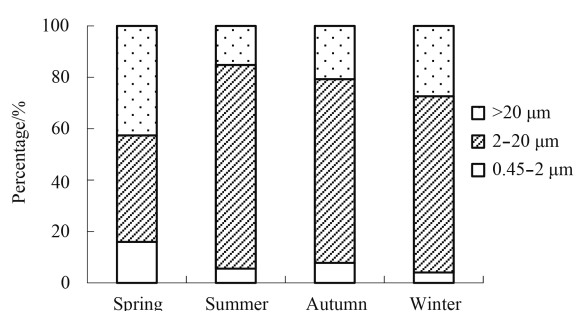
**Fig. 3.** Spatial distribution of Chl *a* ( $\mu\text{g/L}$ ) in the surface water (0.5 m depth) in different season: a. spring, b. summer, c. autumn, and d. winter.

**Table 1.** Seasonal variations of Chl *a* concentration, size-fraction concentration of Chl *a*, seawater transparency and primary production in the Sanggou Bay

Date	Value	Chl <i>a</i> / $\mu\text{g}\cdot\text{L}^{-1}$	<2 $\mu\text{m}/\mu\text{g}\cdot\text{L}^{-1}$	2–20 $\mu\text{m}/\mu\text{g}\cdot\text{L}^{-1}$	>20 $\mu\text{m}/\mu\text{g}\cdot\text{L}^{-1}$	Transparency/m	Primary production/ $\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$
April 2013	mean $\pm$ SD	0.83 $\pm$ 0.45	0.13 $\pm$ 0.11	0.37 $\pm$ 0.31	0.33 $\pm$ 0.19	2.23 $\pm$ 1.22	71.76 $\pm$ 53.30
	range	0.30–2.04	0.00–0.43	0.09–1.28	0.08–0.85	0.60–5.00	17.57–189.63
July 2013	mean $\pm$ SD	6.89 $\pm$ 5.01	0.39 $\pm$ 0.33	5.33 $\pm$ 4.00	1.17 $\pm$ 1.22	1.18 $\pm$ 0.29	579.11 $\pm$ 488.30
	range	0.86–20.46	0.01–1.20	0.67–17.10	0.13–3.29	0.70–1.90	84.56–1 844.46
October 2013	mean $\pm$ SD	0.83 $\pm$ 0.45	0.06 $\pm$ 0.06	0.59 $\pm$ 0.36	0.18 $\pm$ 0.22	0.86 $\pm$ 0.27	47.38 $\pm$ 36.78
	range	0.36–2.00	0.00–0.20	0.21–1.81	0.00–0.96	0.50–1.30	11.67–141.37
January 2014	mean $\pm$ SD	0.44 $\pm$ 0.41	0.01 $\pm$ 0.02	0.32 $\pm$ 0.35	0.11 $\pm$ 0.08	1.88 $\pm$ 0.50	23.01 $\pm$ 15.52
	range	0.10–1.66	0.00–0.07	0.06–1.38	0.00–0.31	1.00–2.80	4.39–70.43

ated there were very significant differences between different seasons for both Chl *a* and primary production ( $p < 0.01$ ). The subsequent post hoc Tamhane's T2 test showed that the values of Chl *a* in summer differed very significantly from the other three seasons ( $p < 0.01$ ).

Figure 4 shows the seasonal variations and contribution of size-fraction phytoplankton in surface seawater of the Sanggou Bay. Results show that the nano-phytoplankton was the most important size-fraction and accounted for about 65.1% of total Chl *a* on average. In contrast, the smaller  $< 2 \mu\text{m}$  size class contributed a mere 8.4% of the total Chl *a*. Taking the seasonal variations into account, nano-phytoplankton was dominant during summer, autumn and winter, whereas nano- and micro-phytoplankton together dominated in spring.

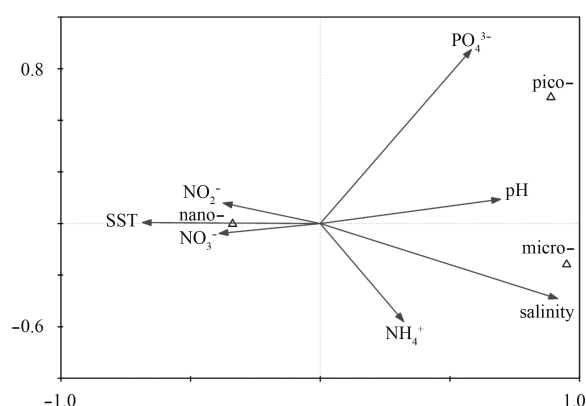


**Fig. 4.** Seasonal variation of the percentage of size-fraction phytoplankton in surface seawater of the Sanggou Bay.

Seasonal variability of different environmental parameters, including SST, salinity, pH and nutrients, in the sea surface of survey area are listed in Table 2. A CCA was performed with seven environmental variables and three phytoplankton fractions (Fig. 5). The eigenvalues of Axis 1 ( $\lambda = 0.054$ ), Axis 2 ( $\lambda = 0.004$ ) and Axis 3 ( $\lambda = 0.009$ ) explain 82.0% of the total data variation. Results show that the nano-phytoplankton fraction was correlated to SST, nitrate and nitrite, whereas micro-phytoplankton fraction was more correlated to salinity and ammonia. The pico-phytoplankton was correlated to phosphate and pH. The Monte Carlo test demonstrated that the correlation between biological and abiotic matrices was statistically significant for all canonical axes ( $p = 0.002$ ).

### 3.2 Contribution of natural plankton to the carbon source of Zhikong scallop *Chlamys farreri*

Water temperature and salinity during the clearance rate experiment ranged from 16.8°C to 17.9°C and from 31.01 to 31.09. Abundance and carbon biomass for the different categories in the natural ambient seawater were presented in Table 3. The concentration of Chl *a* of nano-phytoplankton and micro-phyto-



**Fig. 5.** CCA ordination of size-fraction phytoplankton and environmental parameters in the Sanggou Bay.

plankton was significantly higher than Chl *a* of pico-phytoplankton ( $p < 0.01$ ). The phytoplankton biomass for Chl *a* of nano-phytoplankton and micro-phytoplankton and Chl *a* of pico-phytoplankton was occupied 84.8% and 15.2% of the total biomass respectively. Among the three types of autotrophic and/or heterotrophic plankton, picoplankton and nanoflagellates were the most abundant plankton types with the mean concentration of  $1.51 \times 10^9$  and  $6.20 \times 10^6$  cell/L. The total planktonic carbon biomass was 121.59  $\mu\text{g/L}$  and nanoflagellates constituted the bulk of total plankton biomass (52.4%).

Clearance rates of scallops for each plankton fraction ranged from 0.60 to 7.10 L/(g·h) and increased with the size of plankton (Table 4). Scallops cleared Chl *a* of nano-phytoplankton and micro-phytoplankton at a higher rate than Chl *a* of pico-phytoplankton ( $p < 0.01$ ). Furthermore, clearance of picoplankton by scallops was extremely low compare to clearance of nanoflagellates and ciliates ( $p < 0.01$ ). Based on the clearance rates and carbon biomass, we calculated the contribution of each plankton fraction to the carbon source of scallops in autumn in the Sanggou Bay. The scallops retained much larger amounts of carbon from Chl *a* of nano-phytoplankton and micro-phytoplankton (95.81%) than from Chl *a* of pico-phytoplankton (4.19%). For the planktonic fraction, carbon retained by scallops originated mainly from nanoflagellates ((219.30±40.08)  $\mu\text{g}/(\text{g}\cdot\text{h})$ ) and represent 82.20% of carbon resource, then from picoplankton (15.93%), and finally from ciliates (1.86%).

## 4 Discussion

The physical and chemical properties of a given environment are very important factors controlling the size distribution of phytoplankton (Cermeño et al., 2006). In general, it is accepted that, pico-phytoplankton cells ( $< 2 \mu\text{m}$ ) are dominated in oceanic oligotrophic areas (Zubkov et al., 2000), while in coastal areas

**Table 2.** The data of environmental parameters measured during the sampling periods

Date	Value	SST/°C	Salinity	pH	Nitrate/mg·L <sup>-1</sup>	Nitrite/mg·L <sup>-1</sup>	Ammonia/mg·L <sup>-1</sup>	Phosphate/mg·L <sup>-1</sup>
April 2013	mean±SD	7.80±1.23	30.76±0.46	8.27±0.09	0.173±0.104	0.002±0.001	0.063±0.032	0.011±0.007
	range	6.00–9.60	30.20–31.31	8.14–8.39	0.063–0.383	0.000–0.003	0.012–0.141	0.003–0.022
July 2013	mean±SD	20.55±1.69	29.34±0.52	8.04±0.05	0.197±0.150	0.004±0.006	0.028±0.018	0.004±0.002
	range	17.80–23.30	28.23–30.44	7.94–8.13	0.585–0.027	0.000–0.027	0.007–0.071	0.002–0.009
October 2013	mean±SD	18.87±0.52	29.55±0.14	8.02±0.05	0.448±0.484	0.014±0.008	0.066±0.061	0.002±0.002
	range	17.70–19.60	29.26–29.81	7.95–8.10	0.013–1.463	0.004–0.028	0.006–0.185	0.000–0.005
January 2014	mean±SD	3.50±1.11	30.01±0.34	8.12±0.05	0.273±0.103	0.004±0.003	0.007±0.010	0.001±0.001
	range	1.80–5.70	29.20–30.60	8.05–8.20	0.135–0.462	0.001–0.013	0.000–0.035	0.000–0.006

**Table 3.** Abundance (in cell/L or in  $\mu\text{g/L}$ ), carbon biomass (CB in  $\mu\text{g/L}$ ) for the natural seawater measured in October 2014 ( $n=12$ )

Plankton fraction	Abundance	CB
Chl <i>a</i> of nano-phytoplankton and micro-phytoplankton	1.37±0.06	68.50±8.64
Chl <i>a</i> of pico-phytoplankton	0.15±0.08	12.30±2.08
Picoplankton		
Heterotrophic bacteria	(1.50±0.21)×10 <sup>9</sup>	47.26±10.20
<i>Synechococcus</i>	(0.76±0.19)×10 <sup>6</sup>	0.14±0.03
Picoeukaryotes	(1.17±0.17)×10 <sup>7</sup>	9.77±1.26
Nanoflagellates	(6.20±2.22)×10 <sup>6</sup>	63.72±12.37
Ciliates	(0.15±0.04)×10 <sup>3</sup>	0.70±0.09

where factors influencing the composition and dynamics of the phytoplankton community (nutrient, light availability, predation, among others) are more variable in time and space, nano- and micro-phytoplankton usually dominate for considerable periods of time (Iriarte, 1993). In this study, CCA analysis showed that the concentration of pico-phytoplankton was correlated to phosphate and pH. In the context of the phosphorus-deficient and nitrogen-sufficient trophic condition in the Sanggou Bay (Sun et al., 2007), the size fraction of phytoplankton was most likely controlled by nutrient. There have been many investigations on the size distribution of phytoplankton in areas of the coastal sea of China and importance of different sized phytoplankton was described in those studies. Results show that nano-phytoplankton fraction constituted the most important part in the East China Sea (Huang et al., 2006), southern Yellow Sea (Deng et al., 2008), Jiaozhou Bay (Wu et al., 2004; Pan and Shen, 2009; Sun and Sun, 2012) and Laizhou Bay (Cai et al., 2002). The results of the present investigation indicate that nano-phytoplankton is also the most abundant fraction of the phytoplankton in the Sanggou Bay area and similar to previous reports on the phytoplankton characteristic of coastal sea in China. The Sanggou Bay is famous for its large-scale aquaculture for shellfish and seaweed in northern China. Bivalves (scallop *Chlamys farreri* and oyster *Crassostrea gigas*) are the main cultivated shellfish species. Bivalves are suspension feeders and gain nourishment by filtering suspended particles such as phytoplankton and detritus from the water column, the by-products are dissolved ammonium and bio-deposits of feces and pseudofeces, they are therefore considered “keystone” species which exert “top-down” control of phytoplankton by grazing but also “bottom-up” control through biodeposition and promotion of nutrient removal (Newell, 2004). Previous studies showed that the large-scale bivalves’ aquaculture will affect the phytoplankton community composition (Zhang et al., 2005). Song et al. (2007) pointed out that scallop culture reduced the community diversity of phytoplankton in the Sanggou Bay during 1983–2004. With the development of

aquaculture, the phytoplankton community structure in the Sanggou Bay has significantly changed in the past 30 years (Yuan et al., 2014). Recent studies show that 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 the Sanggou Bay (Zhao et al., 2016). Deep understanding of phytoplankton community structure in the Sanggou Bay will provide guiding information for sustainable management of aquaculture there. Furthermore, it must be pointed out that, limited by the multipoint synchronous size fraction processing technical support, the choice of seasonal sampling with only one date of collection do not appear sufficiently accurate to describe the real phytoplankton dynamics within the bay, especially in case where the high variability being measured during winter and summer surveys. How to integrate the long term and continuous monitoring with temporal and spatial size fraction phytoplankton will be an essential work in the future.

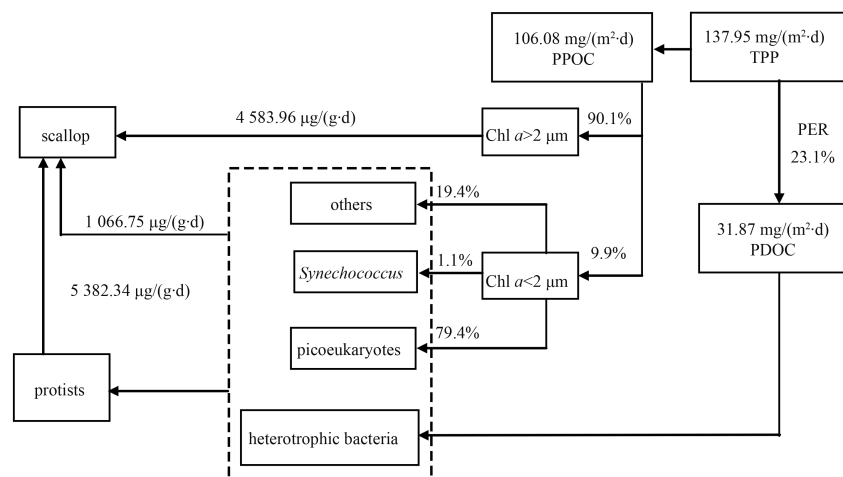
Phytoplankton can produce particulate organic carbon (PPOC) through photosynthesis, while it can also release substantial amounts of dissolved organic carbon (DOC) to the surrounding water (Mague et al., 1980). Most of the past marine production observation were only determined on particulate portion of the primary production. It is well documented that extracellular DOC released from phytoplankton can serve as energy source for microheterotrophic organisms and then is transferred to higher trophic levels (Hansell and Carlson, 2001; Cherrier and Bauer, 2004). A wide variability of percentage of phytoplankton extracellular release (PER) has been reported in works *in situ* (0% to 80%), but the average PER was 20.0% in most cases (Marañón et al., 2004, 2005). Previous studies have shown that phytoplankton-released dissolved compounds are labile and quickly consumed by marine bacteria (Baines and Pace, 1991). Morán et al. (2001) investigated the dependence of heterotrophic bacteria on dissolved organic products released by phytoplankton in the Weddell Sea and Scotia Sea, and the results indicated that phytoplanktonically produced DOC would support more than 90% of the bacterial carbon demand. Roland and Cole (1999) provided an empirical formula to estimate the bacterial carbon demand:  $BGE=0.10+0.68 \times BP/(5.21+BP)$ ,  $BGE=BP/BCD$ , where  $BCD$ ,  $BGE$  and  $BP$  represent bacterial carbon demand, bacterial growth efficiency and bacterial production respectively. We cite the  $BP$  value (125.5 mg/(m<sup>2</sup>·d)) from the results obtained in the Bohai Sea area close to this research area (Xiao and Wang, 2003), it can be estimated that phytoplanktonically produced DOC would support about 19.12% of the bacterial carbon demand. Our  $BCD$  value match with the previous study of Poulton et al. (2016), who found  $BCD$  to be 16% (range 4%–43%) in the Nordic seas of the Arctic Ocean. However, key to the estimation of  $BCD$  is knowledge of the bacterial growth efficiency, which varies consider-

**Table 4.** Clearance rates of scallops for each plankton fraction in October 2014 in the Sanggou Bay

	Abundance		Clearance rate/L·g <sup>-1</sup> ·h <sup>-1</sup>	Carbon retention rate/ $\mu\text{g}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$
	Control	Treatment		
Chl <i>a</i> of nano-phytoplankton and micro-phytoplankton/ $\mu\text{g}\cdot\text{L}^{-1}$	1.15±0.11	0.75±0.04	2.79±0.75	190.99±23.07
Chl <i>a</i> of pico-phytoplankton/ $\mu\text{g}\cdot\text{L}^{-1}$	0.17±0.03	0.15±0.02	0.68±0.25	8.35±1.35
Heterotrophic bacteria/cell·L <sup>-1</sup>	(1.64±0.12)×10 <sup>9</sup>	(1.55±0.10)×10 <sup>9</sup>	0.76±0.38	36.10±7.54
<i>Synechococcus</i> /cell·L <sup>-1</sup>	(0.71±0.04)×10 <sup>6</sup>	(0.66±0.02)×10 <sup>6</sup>	0.60±0.20	0.08±0.02
Picoeukaryotes/cell·L <sup>-1</sup>	(1.05±0.12)×10 <sup>7</sup>	(0.97±0.06)×10 <sup>7</sup>	0.65±0.11	6.32±0.81
Nanoflagellates/cell·L <sup>-1</sup>	(6.17±2.06)×10 <sup>6</sup>	(4.89±1.40)×10 <sup>6</sup>	3.44±1.81	219.30±40.08
Ciliates/cell·L <sup>-1</sup>	(0.10±0.07)×10 <sup>3</sup>	(0.04±0.01)×10 <sup>3</sup>	7.10±2.66	4.97±0.57

ably (7%–69%, [García-Martín et al., 2014](#); 15%–65%, [Wear et al., 2015](#)). Factors determining bacterial growth efficiency may include the taxonomic source and rate of DOC supply, the physiological condition of the bacterial cells, and the ecological pathways of DOC supply ([Fouilland et al., 2014](#); [Wear et al., 2015](#)). This uncertainty in the factors controlling BCD should lead us to develop more long-term and successive measuring technology in the future to improve the precision of the estimation.

In order to better understand the contribution of natural plankton to carbon source of scallop in the mariculture system, we took the autumn as an example and built up a tentative carbon flow schematic diagram (Fig. 6).



**Fig. 6.** A tentative carbon flow schematic diagram in autumn 2014 in the Sanggou Bay (the categories in the dashed box represent the component of picoplankton).

Particle-feeding bivalves rely heavily on their highly modified gills to capture their food. The ciliated surface of the gills creates a current that flows through the gills and the mantle cavity and this current brings in food. In general, capture efficiency increases non-linearly with increasing particle size to a maximum ([Ward and Shumway, 2004](#)). In this study, mean clearance rates of scallops ranged from 2.79 to 7.10 L/(g·h) for plankton  $>2 \mu\text{m}$  (Chl *a* of nano-phytoplankton and micro-phytoplankton, nanoflagellates and ciliates). Clearance of picoplankton was extremely low (ranged between 0.60 and 0.76 L/(g·h)) compared to clearance of nanoplankton and microplankton. This finding was highly in agreement with observations on the clearance rate of pearl oysters *Pinctada margaritifera* ([Fournier et al., 2012](#)) which highlighted the strong relationship between clearance rates and plankton size/biovolumes. Numerous studies have shown that this relationship was explained by the gill structure and especially by the disposition of cirri on gill filaments ([Pouvreau et al., 1999](#)). [Jørgensen \(1989\)](#) reported 90% retention efficiency of  $5 \mu\text{m}$  particles in *Crassostrea virginica* and  $4.5 \mu\text{m}$  particles in *Ostrea edulis*, but only 50% retention efficiency of particles  $2.5 \mu\text{m}$  (*C. virginica*) and  $2 \mu\text{m}$  (*O. edulis*). Undoubtedly, clearance rates were highly related to the scallop sizes. The size of the scallop selected in the experiment was following the natural growth period and we did not measure the variation of size on clearance rates. A more realistic estimate would be further taken into account what the sizes of different cohorts would be during the different seasons. It has been reported that bivalves cannot efficiently retain particles smaller than  $2 \mu\text{m}$  diameter ([Kreeger and Newell, 1996](#)). In this study, although the picoplankton can provide the carbon source of the scallops in the rate of  $1\,066.75 \mu\text{g}/(\text{g}\cdot\text{d})$ , the low con-

tribution (less than 10%) indicate they are only represent a weak carbon resource retained for scallops. The high quantities of carbon resource were from protist ( $5\,382.34 \mu\text{g}/(\text{g}\cdot\text{d})$ ). This result supports the important trophic link of protist between picoplankton and the food resources of scallop. Compared to the ciliates ( $119.23 \mu\text{g}/(\text{g}\cdot\text{d})$ ), scallops retained higher quantities from nanoflagellates ( $5\,263.11 \mu\text{g}/(\text{g}\cdot\text{d})$ ). Similar results were found in the diet research on the pearl oyster *Pinctada margaritifera* in Ahe atoll lagoon, [Fournier et al. \(2012\)](#) found that carbon retained by pearls oysters originated mainly from nanoflagellates (64%), then from dinoflagellates and ciliates (27%), and finally from picoplankton (8%). However, the microbial food web is much more complex than food chain due to the existence of multiple trophic levels within the microbial food web ([Chen et al., 2009](#)). The predator-prey relationship between protist and picoplankton, even protist themselves still unclear and need to be further studied.

Regarding the total primary production (TPP), it can be estimated from the literature data (23.1%) for the percentage of phytoplankton extracellular release (PER) ([Liu, 2012](#)) and the calculation of the photosynthetically produced particulate organic carbon (PPOC) by the chlorophyll method. Results show that scallops obtained carbon source from natural plankton with the rate of  $11\,033.05 \mu\text{g}/(\text{g}\cdot\text{d})$ . Protists (nanoflagellates and ciliates) were the dominant source of carbon retained by scallop (48.78%). The second source of carbon for scallops was Chl *a* of nano-phytoplankton and micro-phytoplankton (41.55%).

## 5 Conclusions

The current results suggest that nano-phytoplankton was the most important size-fraction and accounted for about 65.1% of total Chl *a* in the Sanggou Bay. In autumn, protists (nanoflagellates and ciliates) were the dominant source of carbon retained by scallop. The microbial loop can provide 58.45% of the carbon source for farmed scallops. Microbial loop plays an important role in the marine aquaculture system in the Sanggou Bay.

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