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Selective feeding of bay scallop *Argopecten irradians* on phytoplankton community revealed by HPLC analysis of phytopigments in Bohai Sea, China*

JIANG Tao[1, 2], WANG Longhua[1, 3], ZHANG Fuchong[4], FANG Xiao[4], LU Lin[1], ZHANG Jihong[1, 2], WANG Wei[1, 2], QU Keming[1, **], CHAI Chao[3, **]

[1] *Key Laboratory of Sustainable Development of Marine Fisheries, Ministry of Agriculture and Rural Affairs, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao 266071, China*

[2] *Function Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266200, China*

[3] *Qingdao Engineering Research Center for Rural Environment, Qingdao Agricultural University, Qingdao 266109, China*

[4] *Ocean Fisheries Science Research Institute of Hebei Province, Qinhuangdao 066201, China*

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Abstract Understanding the feeding selectivity on phytoplankton by shellfish is currently a big challenge. In order to investigate the feeding behavior of bay scallop (*Argopecten irradians*) on phytoplankton, we compared its compositions of phytopigments in digestive glands with those in the surrounding seawater, and conducted five consecutive investigations between July and November 2016 in a bay scallop culture area along coast of Qinghuangdao City, northwest of the Bohai Sea, China. Phytopigments in four-size fractionated phytoplankton of seawater (micro- (20–200 μm); nano(L)-[10–20 μm]; nano(S)-[2.7–10 μm], and pico-[<2.7 μm]) and digestive glands of *A. irradians* were examined to investigate the selective feeding of *A. irradians*. Results show that fucoxanthin and peridinin constituted the major part of taxonomically diagnostic carotenoids (TDCs) in the micro- and nano(L)-phytoplankton in seawater. Compared with total phytoplankton biomass of seawater (TPB, sum of the four sizes), a substantial decrease of fucoxanthin proportion to total DCs in digestive glands was observed while that of peridinin, 19′-butanoyloxy-fucoxanthin, alloxanthin and 19′-hexanoyloxy-fucoxanthin showed an obvious increase when those pigments were mainly confined to micro-sized phytoplankton (20–200 μm). However, zeaxanthin and prasinoxanthin were mainly confined to nano(s)- and pico-phytoplankton, of which the proportions in digestive glands were usually lower in TPB. The contribution of lutein to total DCs in digestive glands (with an average of 7.23%) increased compared with TPB of seawater (with an average of 0.63%) during all five sampling times.

Keyword: phytoplankton; pigments; selective feeding; *Argopecten irradians*; aquaculture

1 INTRODUCTION

It is well known that phytoplankton is a main food resource for shellfish aquaculture in the marine environment. However, filter-feeding shellfish do not feed on all phytoplankton species and preferentially select some species for ingestion (Shumway et al., 1985; Ward and Shumway, 2004). The process of feeding selectivity operates on different levels. The first pre-ingestive selection occurs in the ctenidium, which can retain particles. The retained particles are delivered to the labial palps where a second pre-ingestive mechanism occurs. Both selection processes are significantly influenced by the morpho-physical characteristics of the prey, including particle shape and size, morphology, motility, toxicity, nutritional contents, as well as membrane composition (Ward

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** Corresponding authors: qukm@ysfri.ac.cn; chaichao1999@126.com

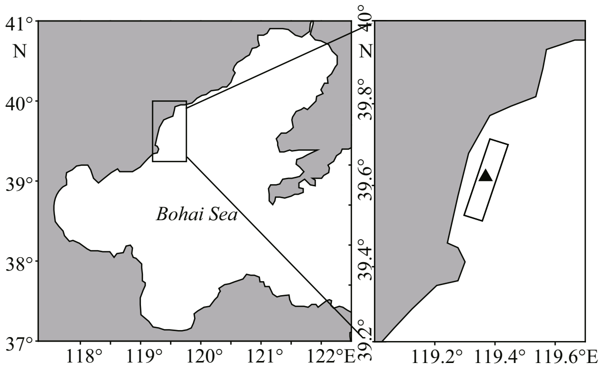

Fig.1 Study site (solid triangle) in the aquaculture zone of scallop *Argopecten irradians* in Qinghuangdao sea area, northwest of the Bohai Sea

and Shumway, 2004; Ren et al., 2006; Safi et al., 2007; Safi and Hayden, 2010; Espinosa et al., 2016; Rosa et al., 2017).

The filtration activity and food preferences of filter-feeding shellfish have been investigated, but the findings are still limited and contradictory (Shumway et al., 1985; Ward and Shumway, 2004; Ren et al., 2006). Previous studies show that bivalves mainly filter larger phytoplankton group (>3 μm), while no concrete conclusions are drawn for their feeding selectivity on different algal species (reviewed by Ward and Shumway, 2004). Several studies have suggested no selective feeding of bivalves on different phytoplankton species due to the similar patterns of phytoplankton composition in the gut of those shellfish and surrounding seawater (Shumway et al., 1987; Kamermans, 1994). In comparison, many studies demonstrated that some diatom species were indigestible or unfavorable food sources for many species of bivalves (Bougrier et al., 1997; Mafra et al., 2009). Furthermore, other studies suggested that some bivalves appear to preferentially ingest dinoflagellates and other flagellated cells compared with diatoms (Shumway et al., 1985; Loret et al., 2000; Safi and Hayden, 2010; Frau et al., 2016). The above-mentioned studies reflect the complexity of the feeding behavior of filter-feeding shellfish.

In dealing with the phenomenon of differential utilization, a comparison of phytoplankton composition between gut contents of bivalves and the water column appears to be a useful method under field conditions (Loret et al., 2000; Frau et al., 2016). Phytoplankton species in the gut contents of shellfish, which is detected using the microscopic method, have generally been used as the indicators for diet composition in previous studies (Rouillon et al., 2005; Frau et al., 2016). However, the microscopic method is time consuming and presents difficulty in identifying fragile and small phytoplankton cells. Phytoplankton pigments, determined by high-performance liquid chromatography (HPLC) method, can provide a qualitative and quantitative analysis of whole phytoplankton community structures in the marine environment (Table S1, Jeffrey and Vesk, 1997). The HPLC pigment analysis can give the reliable results of phytoplankton composition in gut contents of shellfish, especially for those fragile phytoplankton cells, easily deformed or destroyed by digestion process, as well as pico-sized phytoplankton, which can not be identified by microscopy. However, this method has been rarely performed except for the investigation by Loret et al. (2000) and Lavaud et al. (2018). A comparison between gut contents of bivalves and size-fractionated phytopigments of seawater has yet been conducted in previous studies (Loret et al., 2000; Lavaud et al., 2018), which can provide a more accurate knowledge on differential feeding of bivalves on different algal groups from different size classes.

In recent decades, the shellfish aquaculture area in China has increased substantially and China has become the largest shellfish production country in the world (Zhang et al., 2009). Bay scallop *Argopecten irradians* is a major cultured scallop species in China and the industry of bay scallop culture has expanded rapidly since it was introduced from the U.S.A. in 1982 (Zhang et al., 1991). Qinhuangdao sea area is one of the largest bay scallop production sites in China. However, in recent years, this sea area has suffered from an increasing problem of eutrophication and recurrent brown tide blooms caused by pico-sized phytoplankton *Aureococcus anophagefferens*. Under such environmental conditions, the feeding activities and growth of cultivated *A. irradians* were significantly disturbed (Zhang et al., 2012). This study aims to: (1) qualitatively and quantitatively characterize the size-fractionated phytopigments in seawater; (2) investigate the feeding behavior of *A. irradians* on phytoplankton by comparing the composition of phytopigments of their digestive glands with that of the phytoplanktons of the surrounding seawater.

2 MATERIAL AND METHOD

Monthly investigations were conducted between July and November 2016 in scallop culture areas along coast of Qinghuangdao City, northwest of the Bohai Sea (Fig.1), which is one of the most important

Table 1 Physical and biological parameters for *Argopecten irradians* and sea water during the investigation period in aquaculture area of Qinghuangdao

Month	Condition index of bay scallop		Environmental parameters of sea water	
	Mean shell length (mm)	Mean fresh weight (g)	Temperature (°C)	Salinity
07-2016	35.0±3.7	7.1±1.7	26.4	34.0
08-2016	47.4±3.5	14.9±3.1	27.3	33.0
09-2016	56.2±3.1	26.6±4.5	23.0	34.1
10-2016	61.3±3.4	33.2±4.7	14.8	34.3
11-2016	63.6±3.3	34.0±4.9	5.2	34.3

aquaculture areas for *A*. *irradians* in North China. The study site is located in the middle of the aquaculture zone with water depth of about 7 m, approximately 2.5 miles away from shore. Environmental parameters (temperature, salinity) in surface seawater (1.0 m) were recorded by a water quality meter (YSI Inc., Yellow Springs, OH, USA). Surface water was collected at a depth of 1 m (3 L in triplicates for pigments analysis). Seawater samples were pre-filtered through 200-μm mesh to remove larger detritus, macro-algae, and zooplankton. Then 3-L seawater was filtered in sequence through 20 μm (nylon membrane filters, Isopore, Millipore), 10 μm (nylon membrane filters, Isopore, Millipore), 2.7 μm (GF/D, Whatman), and 0.7 μm (GF/F, Whatman) for the size-fractionated pigment analysis. All filters were frozen immediately in liquid nitrogen and stored at -80°C for pigment analysis. Size-fractionated phytoplankton included micro- (20–200 μm), nano(L)- (10–20 μm), nano(S)- (2.7–10 μm), and pico-sized fractions (<2.7 μm) in this study. Total phytopigment in seawater is the sum of the four sizes. Similarly, total phytoplankton biomass (TPB) indicates the sum of the four-size fractions of phytoplankton in seawater.

The seeds of scallop *A*. *irradians* (<10 mm length) were introduced in this aquaculture zone during the end of May 2016 and harvested during November 2016. *A*. *irradians* was suspended in cages in sea surface layer of 0.5–2 m depth. All cages were the same size, and attached to the floating long-line. During each investigation, a cage was randomly selected from the same long-line to assure the scallop samples from the same batch. Forty scallops were randomly picked and measured to determine the mean shell length and mean fresh meat weight. For pigment analysis, three digestive glands of *A*. *irradians* were carefully cut and stored under -80°C until pigment extraction in the laboratory.

Pigment analysis was performed using HPLC-UV method according to Zapata et al. (2000). Filters of seawater were cut into small pieces and phytopigments were extracted by sonication with 3 mL of 95% methanol in an icy bath for 5 min. For scallop samples, 15 digestive glands were homogenized by a tissue homogenizer. Approximately 0.1 g of digestive gland tissue was extracted in 3 mL of 95% methanol, similar to the extraction method of filters. The extract (1 mL) was transferred to a 2-mL screw top vial and diluted with 250 μL Milli-Q water before injection to improve the separation of pigments in the chromatographic column. Twenty-two authentic pigment standards (DHI Inc. Denmark), including chlorophyll *a* (Chl *a*), Chl *b*, Chl *c*2, Chl *c*3, divinyl chlorophyll *a* (DV-Chl *a*), pheophytin *a* (Phe *a*), pheophorbide *a* (Pheide *a*), Mg-2,4-divinylpheoporphyrin (MgDVP), alloxanthin (Allo), 19′-but-fucoxanthin (But), canthaxanthin (Cantha), β,β-carotene (β-Car), diadinoxanthin (Diadino), diatoxanthin (Diato), fucoxanthin (Fuco), 19′-hex-fucoxanthin (Hex), lutein (Lut), neoxanthin (Neo), peridinin (Peri), prasinoxanthin (Pras), violaxanthin (Viola), and zeaxanthin (Zea), were used in this study.

One-way ANOVA was used to evaluate the differences in (1) phytopigment concentrations in fractionated phytoplankton and (2) the proportions of the eight taxonomically diagnostic carotenoids (TDCs) between the seawater and the digestive glands. All data analyses were performed by SPSS 19.0.

3 RESULT

3.1 Environmental parameters and condition index of *Argopecten irradians*

During the study period, water temperature showed the highest value in August (27.3°C) and the lowest in November (5.2°C) (Table 1). Salinity varied slightly from 33.0 to 34.3 with lower values in the summer and early autumn (July–September), during which rainfall was heavy.

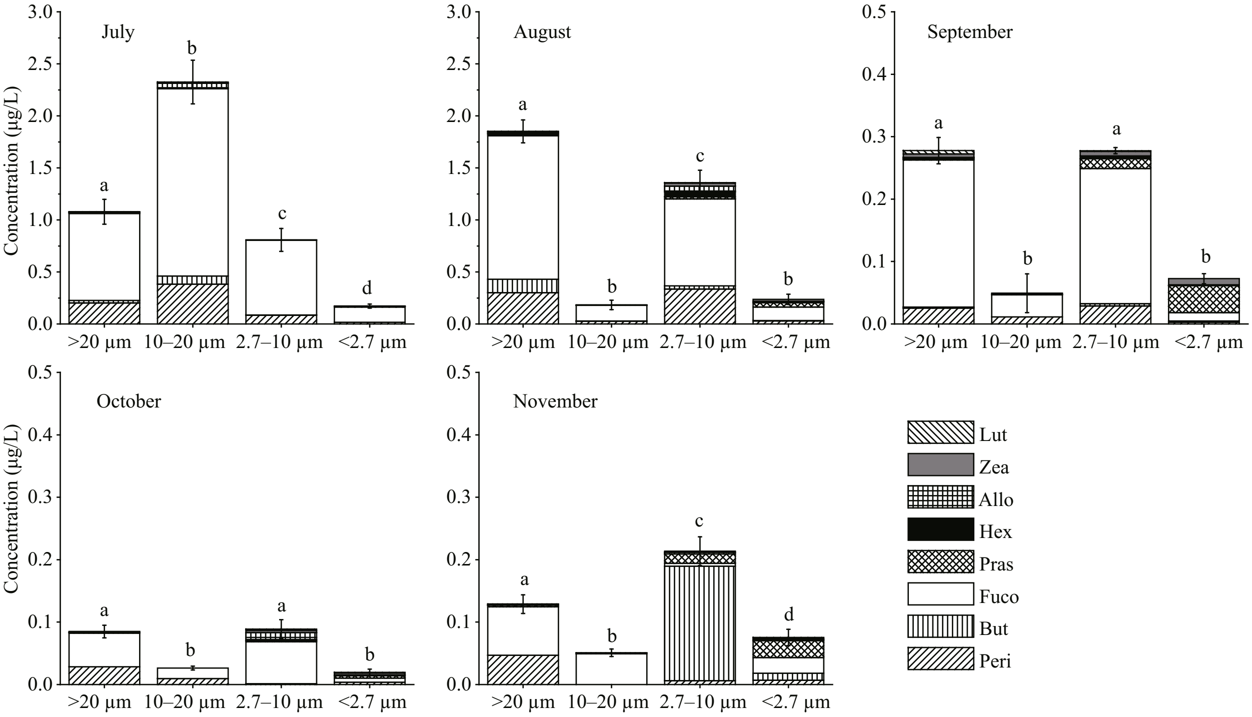

Fig.2 Monthly variations of size-fractionated diagnostic carotenoids in seawater from the aquaculture area of Qinghuangdao

Different letters above the bar indicate significant difference based on one-way ANOVA (Tukey test, $P<0.05$).

Table 2 Size-fractionated Chl *a* in sea water during the investigation periods in aquaculture area of Qinghuangdao (mean values±standard deviation)

Month	Chl *a* (>20 μm)		Chl *a* (10–20 μm)		Chl *a* (2.7–10 μm)		Chl *a* (<2.7 μm)		Total Chl *a*
	Content(μg/L)	Percent (%)	Content(μg/L)	Percent (%)	Content(μg/L)	Percent (%)	Content(μg L)	Percent (%)	Content(μg/L)
07-2016	0.45±0.038[a]	30.98	0.91±0.008[b]	62.23	0.09±0.008[c]	5.84	0.014±0.003[d]	0.96	1.46
08-2016	1.25±0.132[a]	71.90	0.25±0.032[b]	14.28	0.22±0.033[b]	12.39	0.025±0.001[c]	1.43	1.74
09-2016	0.41±0.058[a]	70.31	0.056±0.009[b]	9.56	0.10±0.008[c]	16.55	0.021±0.005[b]	3.58	0.59
10-2016	0.11±0.010[a]	40.82	0.035±0.004[b]	13.11	0.11±0.014[a]	40.07	0.016±0.003[b]	5.99	0.27
11-2016	0.14±0.008[a]	46.98	0.066±0.008[b]	22.15	0.079±0.009[b]	26.51	0.013±0.001[c]	4.36	0.30

Values in the same row having different letters in superscript indicate significant difference based on one-way ANOVA (Tukey test, $P<0.05$).

Argopecten irradians underwent rapid growth from July to September. However, their shell length and tissue growth slowed down between October and November, accompanied by a sharp decrease in temperature and total Chl *a* (Tables 1, 2).

3.2 Size-fractionated phytopigments in the seawater

High concentrations of total Chl *a* were observed at 1.46 and 1.74 μg/L in July and August, respectively (Table 2). Micro- (20–200 μm) and nano(L)-phytoplankton (10–20 μm) occupied more than 80% of the total Chl *a* except for October and November, during which, in addition to the former, nano(L)-phytoplankton (2.7–10 μm) also made up an important constituent. Pico-phytoplankton (<2.7 μm) contributed a minor portion of total Chl *a*, ranging from 0.96% to 5.99% during the whole investigation (Table 2).

Twenty-one pigments were detected in the seawater of the study area (Table S1). In this study, eight taxonomically diagnostic carotenoids (TDCs), including Fuco, Peri, Lut, Zea, Allo, Hex, Pras, and But, were used to analyze the size distribution patterns of different phytoplankton groups in five consecutive investigations (Fig.2). In accordance with Chl *a*, high concentrations of total TDCs occurred in July and August (Table 2; Fig.2). In four size fractions of phytoplankton, the total DC concentration of micro- and nano(s)-phytoplanktons were considerably higher than those of other size-fractions during all the

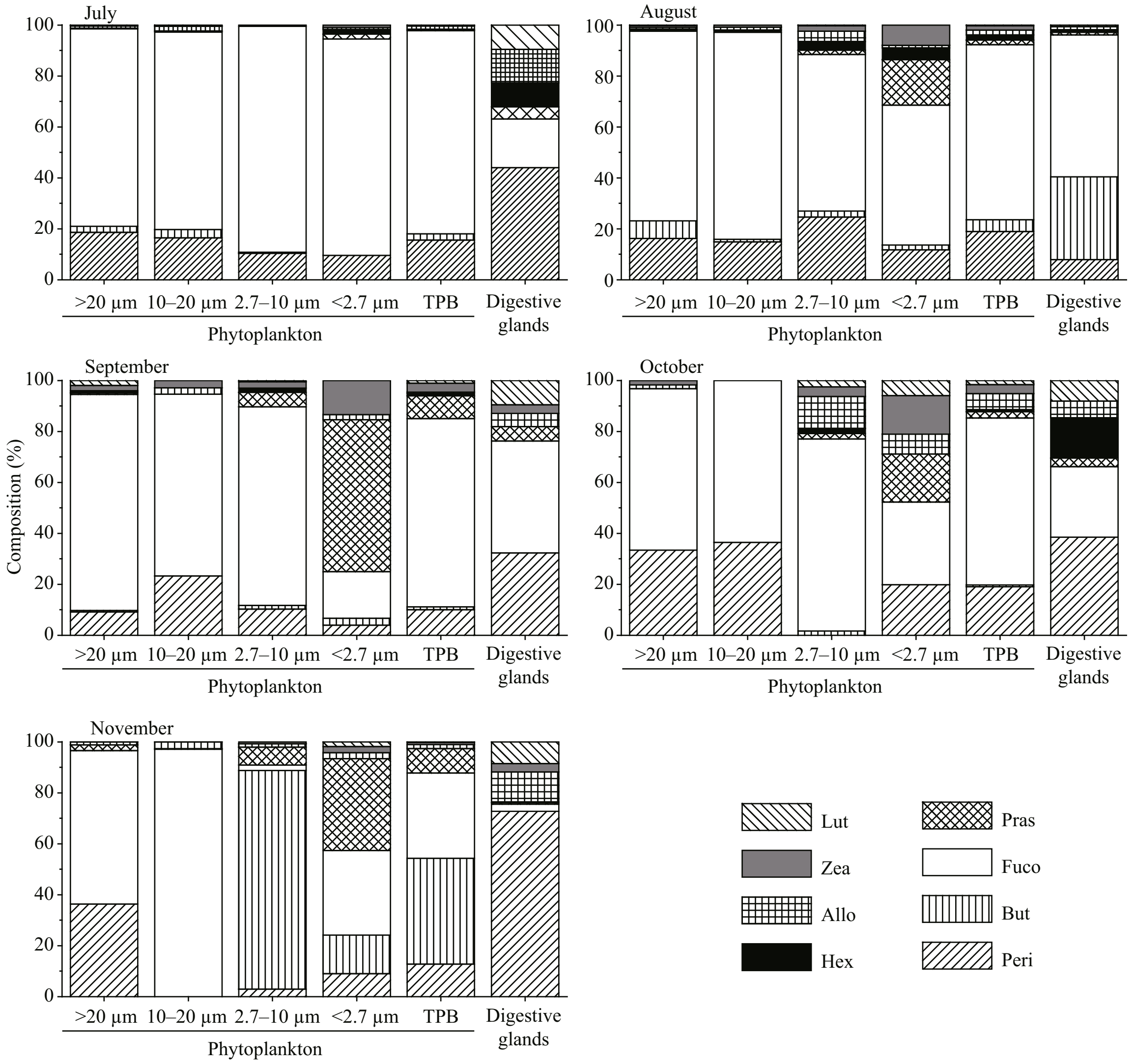

Fig.3 Composition of diagnostic carotenoids in seawater and in the digestive glands of *Argopecten irradians* from the aquaculture area of Qinghuangdao

TPB means total phytoplankton biomass, sum of the four sizes.

investigation periods ($P<0.05$) except for July, when that of nano(L)-phytoplankton was the highest (Fig.2). In the micro- and nano(L)-phytoplanktons, Fuco and Peri constituted the most important part of TDCs (Fig.3). However, contributions of Zea Allo, Hex, Pras, and But to total TDCs increased substantially in nano(L)- and pico-phytoplanktons (Fig.3).

3.3 Comparisons of the pigment compositions between the water and the digestive glands of *Argopecten irradians*

The comparison of the proportions of the eight TDCs between the seawater and the digestive glands showed obvious differences (Fig.3). Compared with TPB (sum of the four-sized phytoplankton) in seawater, a substantial decrease in the Fuco ($P<0.05$) proportion to total TDCs was observed in the digestive glands of *A. irradians* in all sampling times accompanied by an increase in Peri ($P<0.05$) except in August. The proportion of But showed an obvious increase from 4.6% in TPB of seawater to 32.5% in the digestive glands ($P<0.01$) in August. By contrast, it decreased from 41.6% in the former to 0% in the latter ($P<0.01$) in November. But mainly appeared in micro-sized fraction of phytoplankton with a concentration of 0.13 μg/L in August, whereas it was distributed in nano(s)- and pico-sized fractions in November (Figs.2 and 3). Similarly, compared with the TPB of seawater, Allo proportion to total TDCs in the digestive glands significantly increased in July, September, and November when it was mainly distributed in micro- and nano(L)-sized fractions (*P*<0.01) (Figs.2 and 3). Moreover, Hex also showed an increased proportion in the digestive glands in July and October (*P*<0.01; Fig.3). Comparatively, Zea and Pras were mainly confined to nano(s)- and pico-phytoplanktons, of which the proportions in the digestive glands were lower in TPB of seawater (*P*<0.05) with several exceptions, such as Pras in July and Zea in November. The contribution of Lut to total TDCs in the digestive glands (with an average of 7.23%) increased compared with the TPB of seawater (with an average of 0.63%) during all five sampling times.

4 DISCUSSION

To our best knowledge, this study is the first one that compares the composition of phytopigments in digestive glands of bivalves with that of the size-fractionated particulate organic matter (POM) in seawater. Similar studies by Loret et al. (2000) and Lavaud et al. (2018) also compared composition of phytopigments between digestive glands of bivalves and POM of seawater. They concluded that phytopigments were the suitable indicators when trying to infer the selective feeding on phytoplankton by bivalves. However, it is difficult to make clear the size-dependent feeding selectivity of bivalves by Loret et al. (2000) and Lavaud et al. (2018) because some phytoplankton functional groups span a wide-sized spectrum from pico-size to micro-one (Seoane et al., 2006), which also can be shown in the size-fractionated phytopigments by Figs.2 and 3 in this study.

Eight diagnostic carotenoids, including Peri, But, Fuco, Pra, Hex, Allo, Zea, and Lut, were detected in all four size fractions of phytoplankton in seawater during the investigation period (Figs.2 and 3), which showed the presence of at least eight phytoplankton taxa (dinoflagellates, diatoms, prymnesiophyte, chrysophytes, prasinophytes, chlorophytes, cryptophytes, and cyanobacteria) in the aquaculture area of Qinghuangdao (for algal division corresponding to diagnostic pigments, summarized in Table S1). In this study, we mainly focused on the comparison of diagnostic pigment composition between phytoplankton in seawater and digestive glands to reveal the differential feeding of scallop *A. irradians* on phytoplankton community.

Our results support the proposals of Dupuy et al. (2000), which suggest that picoplankton represents a less valuable trophic resource than micro- and nano-phytoplankton for farmed oysters because pico-particles can be less retained. In this study, the lower proportions of Zea, But, and Pras in the digestive glands than those of seawater suggested that *A. irradians* could ingest less pico-phytoplankton than micro- and nano-one (Fig.3). Similarly, nano(S)-phytoplankton were also proven to be less ingested than micro- and nano(L)-phytoplankton by *A.irradians*. For example, But-Fuco was most concentrated in nano(S)-phytoplankton in November, but it was not found in the digestive glands of *A irradians* (Figs.2 and 3). This result might be attributed to the small cell size of But-containing algal species. Previous reports have revealed that oysters can retain more than 50% of >3 µm particles and 100% for 7 µm particles (reviewed by Cranford et al., 2011), which indicated a lower retention rate in the lower end of the nano-sized spectrum of phytoplankton. However, several exceptions, such as more proportion of Pras and Zea in digestive glands than in TPB of seawater during July and November, respectively, were observed in this study. This result suggests that pico-phytoplankton may also become easily available to the bivalves through particle aggregation processes or through linkage to higher tropic levels through the micro-zooplankton (Cranford et al., 2011). This contrasts with Lavaud et al. (2018) who found Zea remained very low (close to zero for most samples) in digestive tract contents of *Pecten maximus* in spite of high levels in seawater.

Previous studies show that bivalves can preferentially select phytoplankton for filtration, which is significantly influenced by cell size (Safi et al., 2007; Safi and Hayden, 2010; Cranford et al., 2011). Cranford et al. (2011) summarized that bivalves can efficiently retain the particles ranging from more than 2 µm to 8 µm, depending on various bivalve species. Consequently, picoplankton tends to thrive in shellfish aquaculture areas because their predators (e.g., ciliates and hetero-flagellates) and competitors (e.g., larger diatoms) for nutrients are removed by shellfish filtration (Cranford et al., 2011; Jiang et al., 2016). The dominance of picoplankton can function as an index of food resource depletion by shellfish culture (Safi and Gibbs, 2003; Cranford et al., 2008, 2011; Jiang et al., 2016). In our study area, the concentrations of pico-Chl *a* were relatively low and contributed to 0.96% to 5.99% of total Chl *a* during the investigation period (Table 1), which indicated that stocking density of scallop *A. irradians* did not disturb the size distribution of phytoplankton in this area.

In addition to size-selective feeding, bivalves also prefer to feed on some types of phytoplankton because of their different qualitative factors, such as cell shape, flexibility, stickiness, and swimming ability (see review by Ward and Shumway, 2004). Laboratory studies showed that the European oyster, *Ostrea edulis*, preferentially filtered the dinoflagellate named *Prorocentrum minimum* compared with the similar-sized diatom named *Phaeodactylum tricornutum* and flagellate named *Chroomonas salina* (Shumway et al., 1985). Bougrier et al. (1997) shows that oysters preferentially rejected three relatively small diatoms, such as *S. costatum*, *C. calcitrans*, and *Nitzschia* sp. *closterium*, in pseudo-feces when fed five diets composed of a combination of three to four species from different taxa. Moreover, oysters significantly reduce their clearance rate when fed with *Pseudo-Nitzschia* sp. *multiseries* in unialgal suspensions whether it is toxic or not (Mafra et al., 2009). Field studies have suggested that specific phytoplankton can be selectively cleared from natural water containing a wide range of particle size and a mixture of various phytoplankton species (Wetz et al., 2002; Frau et al., 2016; Jiang et al., 2016). Wetz et al. (2002) demonstrated preferential feeding by oysters on phototrophic nanoflagellates but not on heterotrophic nanoflagellates and cyanobacteria, using naturally occurring microbial assemblages in tidal creeks of South Carolina, USA. In the oyster aquaculture area of Daya Bay, South China Sea, the depletion of Peri and Hex was higher than that of the other carotenoids, which indicated that flagellated cells might be selectively filtered by oysters (Jiang et al., 2016). Furthermore, comparing phytoplankton composition between gut contents and surrounding seawater appears to provide more concrete evidence of selective feeding by bivalves (Sidari et al., 1998; Loret et al., 2000; Rouillon et al., 2005). Sidari et al. (1998) found that *Mytilus galloprovincialis* fed selectively on dinoflagellates rather than on diatoms by comparing phytoplankton species between seawater and mussel stomachs. Similarly, Rouillon et al. (2005) reported that dinoflagellates contributed 25% to 30% of total phytoplankton abundance in mussel stomachs, whereas they only constitute a minor component in seawater. In this study, differential feeding on flagellates by bivalves is further evidenced by an obvious increase in Peri and Allo (as well as But in August and Hex in July and October) in digestive glands. Peri, which particularly constituted 15.3% of TDCs in water samples, reached 39.1% in digestive glands on the average (Fig.3). The results of this study also corroborate the observations by Loret et al. (2000) and Lavaud et al. (2018), who found an increase in proportions of Peri in the gut of oysters and scallops when compared with that of seawater. However, the results of selective particle clearance for phytoplankton species are difficult to explain considering the current knowledge of suspension feeding in bivalves (Ward and Shumway, 2004). Understanding the effects of different phytoplankton species on the feeding activity of bivalves is a fertile area for future research.

The reasons for the substantially increased proportion of Lut to total TDCs in digestive glands compared with TPB of seawater were unclear in this study. Lut showed the lowest proportion to the total eight TDCs in TPB of seawater. The size distribution of Lut was mainly confined to micro- and nano(S)-phytoplanktons between July and September and pico-phytoplankton during October and November (Fig.3). This study suggested that, in addition to micro- and nano-phytoplanktons, *A. irradians* might utilize filamentous chlorophyta (containing Lut) and its detritus adhering to the cages even the shells, which were removed by the 200-μm mesh and not determined for pigment analysis in this study (see Section 2). This result is especially evidenced when Lut was confined to pico-phytoplankton in October and November. Filamentous algae and its detritus are important food sources for some freshwater bivalves, such as dreissenids and unionids (Bontes et al., 2007; Makhutova et al., 2013). Further study should focus on a combination of microscopy and HPLC method to reveal whether filamentous chlorophyte exist in the digestive gland of *A. irradians*.

However, cautions must be taken when diagnostic pigments are used to track food sources and explain selective feeding behavior of shellfish. Previous studies showed that decay rates of different pigments were slightly different under the natural marine and lake environment (Repeta and Gagosian, 1987; Bianchi and Findlay, 1991; Leavitt and Hodgson, 2001). Leavitt and Hodgson (2001) have summarized that Fuco and Peri belong to the most labile pigment compounds due to the presence of 5,6-epoxides in their chemical structures (Bianchi and Findlay, 1991). Although But and Hex have been seldom mentioned in previous studies (reviewed by Leavitt and Hodgson, 2001), the chemical structure similar to Fuco may suggest that both pigments have similar chemical stability. By contrast, Allo, Lut, and Zea are more stable than Fuco and Peri (Leavitt and Hodgson, 2001). If the relative degree of chemical stability of TDCs in natural sediments suggested by Leavitt and Hodgson (2001) can be applied to digestive glands in this study, an increased proportion of Peri, But, and Hex to total TDCs may indeed suggest preferential digestion of dinoflagellates, prymnesiophyte, and chrysophytes by scallop *A. irradians*. However, the reasons for the elevated Allo proportion in digestive glands observed in this study and by Loret et al. (2000) might be ascribed to its stable chemical characteristics and/or selective feeding on cryptophytes. Previous studies reported selective preservation of Allo in marine sediments (review of Leavitt, 1993; Jiang et al., 2017). Further study should be conducted to investigate the chemical stability and preservation of different phytopigments in the gut of shellfish.

5 CONCLUSION

In this study, Fuco and Peri constitute the most important part of diagnostic carotenoids (DCs) in the micro- and nano(L)-phytoplankton, which suggest that diatoms and dinoflagellates are mainly distributed in micro- and nano(L)-size fractions. Comparatively, the proportion of Zea (cyanobacteria) and Pras (prasinophytes) mainly appeared in nano(S)- and pico-phytoplanktons. Moreover, size of the other DCs (Lut, Allo, Hex and But) varied depending on different months. The comparison of the composition of total DCs between the phytoplankton in seawater and digestive glands of *A. irradians* shows obvious differences, which suggests *A. irradians* can preferentially select phytoplankton for ingestion. The lower proportions of Zea and Pras in the digestive glands than those of seawater propose that *A. irradians* poorly ingests pico-phytoplankton. This study demonstrates the differential feeding on flagellates by *A. irradians*, which is evidenced by an obvious increase in Peri and Allo (as well as But in August and Hex in July and October) in digestive glands. It is worthwhile to note that the contribution of Lut to total DCs in the digestive glands increases compared with the TPB of seawater during all five cruises. This study suggests that, in addition to micro- and nano-phytoplanktons, *A. irradians* might utilize filamentous chlorophyta (containing Lut) and its detritus. In summary, this study presents an interesting mechanism of selective feeding of *A. irradians* on microsized flagellates.

6 DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Electronic supplementary material

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