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1	The effects of different environmental factors on biochemical composition of particulate
2	organic matters in Gwangyang Bay, South Korea
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Abstract

Biochemical composition of particulate organic matter (POM) through phytoplankton photosynthesis is important to determine food quality for planktonic consumers as well as physiological conditions of phytoplankton. Major environmental factors controlling for the biochemical composition were seasonally investigated in Gwangyang Bay which has only natural conditions (e.g., no artificial dams) in South Korea. Water samples for the biochemical compositions were obtained from three different light depths (100%, 30%, and 1%) mainly at 3 sites in Gwangyang bay from April 2012 to April 2013. Different biochemical classes (carbohydrates [CHO], proteins [PRT], and lipids [LIP]) were extracted and then the concentrations were determined by the optical density measured with a spectrophotometer. The highest and lowest of PRT compositions among the three biochemical classes were in April 2012 (58.0%) and August 2012 (21.2%), whereas the highest and lowest LIP compositions were in August 2012 (49.0%) and April 2012 (24.8%), respectively. CHO composition was recorded high in January 2013 and maintained above 25% during the study period. The calorific contents of food material (FM) ranged from 1.0 Kcal m⁻³ to 6.1 Kcal m⁻³ (annual mean \pm S.D. = 2.8 Kcal m⁻³ \pm 1.1 Kcal m⁻³). Based on Pearson's correlation coefficient analysis, a major governing factor for biochemical composition of POM was dissolved inorganic nitrogen loading from river-input in Gwangyang bay. In conclusion, relatively larger amount of FM and higher calorific contents of POM found in this study compared to other regions reflected good nutritive conditions for sustaining productive shellfish and fish populations in Gwangyang bay. Continuous observations are needed for monitoring marine ecosystem response to potential environmental perturbations in Gwangyang bay.

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Key words:

37 Particulate organic matter, biochemical composition, phytoplankton, nitrogen source

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1. Introduction

Particulate organic matter (POM) mostly from phytoplankton photosynthesis in the euphotic layer is an important food source for planktonic consumers in water columns (Cauwet, 1978) and their biochemical contents reaching the benthic environments are largely utilized by benthic organisms (Nelson and Smith, 1986; Rice et al., 1994). Therefore, POM is an essential link between surface and benthic ecosystems (Graf, 1992). Previous studies showed that the biochemical composition of the POM such as protein (PRT), lipid (LIP) and carbohydrate (CHO) levels could provide useful information on the nutritional value which is potentially available to consumers (Mayzaud et al, 1989; Navarro et al., 1993; Navarro and Thompson, 1995). However, previous studies mainly focused on the occurrence in the different patterns of biochemical composition of POM. It is noteworthy to investigate how biochemical composition of POM responds to changes in various environmental factors, such as nutrients, light, temperature, and salinity and to assess food quantity for higher trophic levels.

The coastal areas represent one of the world's most vital aquatic resources, supporting and providing food resources and habitats for large numbers of fish and shellfish species (Kwak et al., 2012; Wetz and Yoskowitz, 2013; references therein). In Gwangyang bay, the southern coast of Korea (Fig. 1), coastal fisheries and shellfish farming have been prevalence. Over the past decades, the bay have become industrialized such as the construction of steel mill company, power plant and industrial complex and environmental disturbances have been predicted. Also, estuaries have a high short-term variability depending on many episodic events, such as freshwater inputs, tidal cycles (neap-spring), and wind (storms) (Cloern and Nichols, 1985). These anthropogenic forces and environmental changes drastically affect the estuarine habitat properties which can cause different biochemical compositions of POM. Unfortunately, little information is yet available on the biochemical composition of POM in the bay, South Korea. Hence, this study tested the question of the main environmental factors determining seasonal variation and of biochemical composition POM and

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assessed quantity of food material (FM) in the bay. Physical (temperature, salinity, irradiance, riverinput and rainfall data), chemical (nutrients), and biological (chlorophyll-a [chl-a], particulate organic carbon [POC] and nitrogen [PON]) parameters were measured in order to both characterize the origin of POM and understand their effects on the biochemical composition of POM. The aims of this study were to: (1) investigate seasonal variation of biochemical composition of POM, (2) identify the origin of POM, and (3) determine a major governing environmental factor for biochemical composition of POM.

2. Materials and methods

2.1. Study site and sampling procedure

The study site was located in Gwangyang Bay (34.9 $^{\circ}$ N, 127.8 $^{\circ}$ E), the southern coast of Korea (Fig. 1). The total area of the bay is 230 km² at mean sea level (Kang et al., 2003). The bay is characterized by semidiurnal tides with a maximal range of about 4.8 m at spring tides (Korea Hydrographic and Oceanographic Administration). Freshwater flows into the bay from the Seomjin River at the northern part of the bay (mean flow 27 m³ s⁻¹ and annually 1.9 x 10⁹ t during the study period; the National Institute of Environmental Research) and seawater enters through the narrow southern channel (Yeosu Channel).

To obtain data for seasonal variation of POM in the euphotic depth, the field samplings were undertaken at 3 stations of the bay (St.1 or St. 2A, St. 4, and St. 5; see Fig. 1) on a seasonal basis April, June, August, and October in 2012 and January and April in 2013. St. 1 was changed to St. 2A after April 2012 because of logistic problems. Both stations have similar environmental conditions at a relatively close distance. Using a 5 L Niskin water sampler, water samples were collected at different depths of 3 light intensities (100%, 30%, and 1% of surface irradiances; hereafter 3 light depths) which were determined by a secchi disk and transferred to brown sample bottles which were previously washed with a solution of 0.1 N HCl. To obtain *in situ* physical parameters, water

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temperature and salinity were measured with YSI-30 (YSI incorporated) and photosynthetically active

88 radiation (PAR) was measured by a quantum sensor (LI-190SA, LI-COR) with a data logger (LI-1400,

89 LI-COR). Rainfall and river input data during the study period were obtained from the Korea

Meteorological Administration (http://www.kma.go.kr/index.jsp) and the National Institute of

91 Environmental Research (http://water.nier.go.kr/main/mainContent.do).

2.2. Chl-a and major inorganic nutrient analysis

In order to determine chl-*a* concentration, water samples from 3 light depths were filtered through 25 mm GF/F (Whatman, 0.7 μm) which were kept frozen immediately and returned to the laboratory at Pusan National University, Korea for a further analysis. The filters for chl-*a* concentration were extracted in 90% acetone in a fridge (4 °C) for 24 h and centrifuged for 20 minutes at 4000 rpm. Using a fluorometer (Tuner Designs, 10-AU) which had been calibrated with commercially purified chl-*a* preparations, chl-*a* concentrations were measured and calculated (Parsons et al., 1984). Water samples for inorganic nutrient concentrations from surface and bottom waters were obtained from Niskin bottles. The samples were kept frozen (-70 °C) and sent for analysis to the laboratory in the East Sea Fisheries Research Institute (QUAATRO, Seal Analytical).

2.3. Particulate organic carbon and nitrogen analysis

The water samples were filtered through pre-combusted (450 °C) 25 mm GF/F (Whatman, 0.7 μ m). The filters for POC, PON, and δ^{13} C values were preserved frozen (-20 °C) and determined using a Finnigan Delta + XP mass spectrometer at the stable isotope laboratory of the University of Alaska Fairbanks, USA.

2.4. Biochemical composition analysis

The water samples for the biochemical composition (carbohydrates, proteins, and lipids) of POM were collected from 3 light depths. The water samples were filtered through 47 mm GF/F

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(Whatman, 0.7 μm pore), which were immediately frozen at -70 °C and preserved for biochemical
composition analysis at the home laboratory.

Protein analysis

Protein (PRT) concentrations were assessed according to a modified method of Lowry at el. (1951). The filters for PRT analysis were transferred into 12 mL centrifuge tubes with 1 mL DH₂O, respectively. The filters were grounded (using a glass rod) in the tubes with a 5 ml alkaline copper solution (a mixture of 2% Na₂CO₃ in 0.1 N NaOH with 0.5% CuSO₄·5H₂O in 1 % sodium or potassium tartrate; 50:1, v/v). The solutions for PRT concentrations were mixed well (using a vortex) and allowed to stand for 10 min at room temperature in the hood. After 10 min, 0.5 mL of diluted Folin-Ciocalteu phenol reagent (1:1, v/v) was added into the solution, mixed occasionally with a vortex mixer, and allowed to sit for 1 h 30 min. The solutions with a blue color were centrifuged at 3,000 rpm for 10 min. Absorbance of the supernatant was measured at 750 nm. Bovine Serum Albumin (2 mg mL⁻¹, SIGMA) was used as a standard for the PRT concentration.

Lipid analysis

Lipid (LIP) concentrations were extracted according to a column method modified from Bligh and Dyer (1959), and Marsh and Weinstein (1966). The filters for LIP analysis were transferred into 16 mL glass tubes with 3 mL of chloroform-methanol (1:2, v/v). The filters in the tubes were grounded, and then the mixtures were mixed using a vortex mixer. For LIP extraction, glass tubes with samples were stored in the fridge (4 °C) to prevent the solvents from evaporating. After 1 h, the solvents were centrifuged at 2,000 rpm for 10 min and the supernatants were collected and stored in new tubes. This extraction procedure was performed once again immediately. When the extractions were completed, 4 mL of DH₂O was added to the solution in the new tubes, and the solution was homogenized using a vortex mixer. After mixing, the tubes were centrifuged at 2,000 rpm for 10 min, and the solvents were separated into two phases (the chloroform phase for lipids and methanol +

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DH₂O phase). The methanol + DH₂O phase was removed from the solvent using a Pasteur pipette. The chloroform phase was placed in a dry oven at 40 °C for 48 h. After it totally dried for carbonization analysis (Marsh and Weinstein 1966), 2 mL of H_2SO_4 was added to the tubes and they were placed in a heating block at 200 °C for 15 min. After this heating procedure, the tubes were quickly placed in a water bath at room temperature; 3 ml of DH_2O was added to the tubes and the solvents were homogenized (with a vortex mixer) and stood for 10 min or until all bubbles had disappeared. Absorbance of the supernatant was measured at 375 nm. Tripalmitin solutions were used as a standard for the LIP concentration.

Carbohydrate analysis

Carbohydrate (CHO) concentrations were measured according to Dubois et al. (1956). The POM samples for carbohydrate analysis were transferred individually into 15 mL polypropylene (PP) tubes. After 1 mL of DH₂O was added to the PP tubes, the samples were grounded using a glass rod. One ml of 5 % phenol for CHO extraction was added additionally, and the solutions were allowed to stand for 40 min at room temperature in the hood. After the extraction, 5 mL of sulfuric acid (H₂SO₄) was added to the solutions, mixed using a vortex mixer, and allowed to stand for 10 min. The solutions with an orange-yellow color were centrifuged at 3,500 rpm for 10 min. Absorbance of the supernatant was measured at 490 nm using UV spectrophotometer (Labomed, Germany). D (+) - glucose solutions (1 mg mL⁻¹, SIGMA) were used as a standard for the CHO concentration.

2.5. Statistical analyses and calorific value calculation

Statistical tests were carried out using the statistic software "SPSS" (t-test, ANOVA and Pearson's Correlation Coefficient). The level of significance was set at p < 0.05. The calorific value (Kcal g⁻¹) of the food material (FM) (FM was defined by Danovaro et al. (2000); PRT + LIP + CHO concentrations; hereafter FM) and the calorific content of FM (Kcal m⁻³ = Kcal g⁻¹ × g FM m⁻³) were calculated using the Winberg (1971) equation.

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3. Results

3.1. Seasonal distribution and variation of environmental factors and chl-a concentrations

160 The values of environmental factors were summarized in Table 1. The temperature ranged 161 from 5.5 to 26.1 °C. The temperature increased from April to August (the highest temperature in 162 August 2012 at St. 4: 26.1 °C) and decreased from August to January (the lowest temperature in 163 January 2013 at St. 2A: 5.5 °C). The salinity ranged from 14.5 to 32.9 %. Generally, the salinity 164 increased from St. 1 or St. 2A to St. 5. Relatively lower salinity, which is mainly affected by fresh water input from the Seomjin River, was observed at St. 2A. The annual average euphotic depth was 165 166 6.5 ± 3.4 m, ranging from 2 to 12 m. 167 The highest nutrient concentrations were measured in April 2012, when the concentrations of 168 NO₂ + NO₃, SiO₂, NH₄, and PO₄ were above 5.0 μM, 2.0 μM, and 0.2 μM, respectively, except at 1% 169 light depth at St. 4. All inorganic nutrients except SiO₂ were nearly depleted in August 2012 (Table 1). 170 During the rest of our study period, NO₂ + NO₃ and SiO₂ concentrations were observed with similar 171 decreasing patterns from St.1 or St. 2A to St. 5. NH₄ concentrations averaged from October 2012 to 172 April 2013 were 1.1 μ M \pm 0.4 μ M, ranging from 0.5 μ M to 1.9 μ M. PO₄ concentrations (average \pm 173 S.D. = $0.1 \pm 0.1 \,\mu\text{M}$) ranged from 0 to 0.4 μM throughout the water columns at all stations except at St. 2A in April 2012 during the study period. 174 175 Monthly rainfall and river-input in the study location ranged from 15.6 mm to 559.0 mm 176 (annual mean \pm S.D. = 151.0 mm \pm 155.5 mm) and 42.3 to 447.2 x 10^6 t (annual mean = 144.4 x 10^6 t), 177 respectively. Rainfall and river-input were recorded as high during summer and low during winter 178 (Table 2). Average irradiance during our incubation hour ranged from 167.9 ± 133.5 to 1593.3 ± 414.5 μ mols m⁻² s⁻¹ (average \pm S.D.) from April 2012 to April 2013. The highest and lowest irradiance were 179 180 recorded in April 2013 and April 2012, respectively.

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study period (annual mean \pm S.D. = 3.4 μ g L⁻¹ \pm 2.8 μ g L⁻¹; Table 1). There were no significant differences of chl-a concentrations among 3 light depths and spatial distribution. However, there was seasonal variation of chl-a concentrations during study period. Chl-a concentrations were increased from April to August and decreased from August to October in 2012 and increased slightly again in January and April 2013.

3.2. δ^{13} C values and carbon to nitrogen ratios of POM

 δ^{13} C values of sea surface POM ranged from - 23.1 % to - 16.5 % and the annual average δ^{13} C value was -20.9 % (S.D. = \pm 3.2 %). The annual average carbon to nitrogen (C:N) ratio of POM was 7.0 \pm 0.4 (average \pm S.D.), ranging from 6.8 to 7.7 (Table 3).

3.3. Seasonal variation of biochemical composition

The contents of CHO, PRT, and LIP of POM in the water column ranged from $14.2~\mu g~L^{-1}$ to $412.3~\mu g~L^{-1}$ ($129.5~\pm~87.2~\mu g L^{-1}$), from $22.8~\mu g~L^{-1}$ to $382.4~\mu g~L^{-1}$ ($155.0~\pm~73.3~\mu g L^{-1}$), and from $21.4~\mu g~L^{-1}$ to $401.4~\mu g~L^{-1}$ ($154.9~\pm~78.9~\mu g L^{-1}$), respectively (Table 4). The FM contents of POM ranged from $170.9~\mu g~L^{-1}$ to $915.7~\mu g~L^{-1}$ ($435.5~\pm~175.5~\mu g L^{-1}$). Since there were no significant differences in biochemical concentrations of POM and FM among 3 light depths and spatial distributions, we averaged each biochemical compound and FM on monthly basis. The CHO and LIP concentrations increased from April to August and decreased from August to October in 2012. In contrast, the PRT concentrations decreased from April to October in 2012 and increased from October in 2012 to April in 2013. The seasonal pattern of FM concentrations was similar to the pattern of chl-a concentrations.

In order to estimate the biochemical composition as food quality, we obtained relative contributions of each biochemical concentration of POM to FM, based on percentage basis. The biochemical composition of each class (CHO, PRT and LIP) ranged from 8.3% to 59.1%, from 6.8% to 74.9% and from 9.4% to 68.3%, respectively (annual mean \pm S.D. of CHO, PRT, and LIP

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composition = $26.4 \pm 9.4\%$, $37.8 \pm 16.1\%$, and $35.7 \pm 13.9\%$, respectively; Table 4). We found the seasonal variation of biochemical composition based on monthly basis of biochemical composition (Fig. 2). To illustrate these variations of biochemical composition of POM, the highest and lowest PRT compositions were in April 2012 and August 2012. In contrast to PRT compositions, the highest and lowest LIP compositions were in August 2012 and April 2012. CHO composition was recorded high in January 2013, but to compare CHO composition to PRT and LIP composition, CHO composition was not strong varied during the study period.

3.4. Seasonal variations of the calorific values and contents of FM

The calorific values and contents of FM ranged from 5.4 Kcal g^{-1} to 7.9 Kcal g^{-1} (annual mean \pm S.D. = 6.6 Kcal $g^{-1} \pm 0.6$ Kcal g^{-1}) and 1.0 Kcal m^{-3} to 6.1 Kcal m^{-3} (annual mean \pm S.D. = 2.8 Kcal $m^{-3} \pm 1.1$ Kcal m^{-3}), respectively (Table 4). The calorific values of FM had no apparent seasonal pattern, whereas the calorific contents of FM had a seasonal pattern similar to the seasonal variation of FM concentrations.

3.5. Relationship between biochemical pools and environmental conditions

220 Relationships between biochemical pools and environmental conditions were performed 221 using Pearson's correlation matrix. Based on the results, we found a significant, positive relationships 222 between PRT composition and river-input (r = 0.84, p < 0.01, Table 5, Fig. 3) and PRT composition 223 and dissolved nitrogen concentrations (NH₄: r = 0.69, p < 0.01; NO₂+NO₃: r = 0.54, p < 0.01, Table 224 5). Lipid composition had an inverse relationships with river-input (r = -0.63, p < 0.01) and dissolved 225 nitrogen concentrations (NH₄: r = -0.59, p < 0.01; NO₂+NO₃: r = -0.53, p < 0.01). These 226 relationships led to a significant reverse relationship between PRT composition and LIP composition 227 (r = -0.81, p < 0.01, Fig. 4). PRT composition was negatively correlated with temperature (r = -0.52, p = 0.01, p < 0.01, Fig. 4). 228 < 0.01), whereas LIP composition was positively correlated with temperature (r = 0.72, p < 0.01). There were no significant relationships between PRT composition and irradiance and LIP composition 229

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and irradiance.

4. Discussion

4. 1. Environmental conditions and chl-a concentration

The annual average chl-a concentration during the research period was 3.4 μ g L⁻¹ (S.D.= $\pm 2.8 \ \mu$ g L⁻¹) with a range from 0.8 to 14.2 μ g L⁻¹ which is in a similar range of chl-a concentrations reported previously in Gwangyang bay, although it varied across different seasons and sampling depths (Cho et al., 1994; Choi et al., 1998; Lee et al., 2001; Kwon et al., 2001; Jang et al., 2005; Yang et al., 2005; Beak et al., 2011; Min et al., 2011; Beak et al., 2015). Previous studies reported that chl-a concentration was influenced mainly by salinity, temperature, and nutrients (nitrate and phosphate) depending on freshwater input from the Seomjin River. Our results in this study were similar to former studies (r = 0.34 and -0.41, p < 0.05, n = 48 and 28 for salinity and NH₄, respectively). However, high chl-a concentrations were previously recorded in spring and fall, whereas the highest concentrations were observed in summer (August 2012) from this study. In fact, Baek et al. (2015) reported that high chl-a concentrations were found in summer similarly, although there was difference between environmental factors and chl-a concentrations as compared with our results. The high levels of chl-a were observed with high nutrient concentrations and low salinity levels in the surface water by Baek et al. (2015), whereas the high values existed with low nutrient concentrations and high salinity levels in our results.

Despite this dissimilarity of environmental factors with high chl-a concentrations, we also found the highest chl-a concentrations observed in summer. According to Shaha and Cho (2009), there is a tendency with increasing precipitation and river-input in Gwangyang Bay during summer. This trend could increase loading nutrients from freshwater for maintaining phytoplankton growth in summer. In addition, a strong light intensity during summer could be favorable for phytoplankton growth since our study area was extremely turbid conditions during almost all seasons due to

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freshwater discharge and a strong spring-neap tidal oscillation. As a result, the combination of these factors is believed to enhance chl-a concentration and primary production of phytoplankton during summer in Gwangyang Bay.

4. 2. POM characterization

In general, POM consists of a mixture of living as well as detritus materials (phytoplankton, bacteria, zooplankton, fecal pellets, terrestrial matters, etc.) originating from freshwater and estuarine and marine environments. POM samples can be characterized or determined for source of the major contributor(s). The C:N ratio generally ranges between 6 and 10 for phytoplankton, whereas the ratios are between 3 and 6 for zooplankton and bacteria (Savoye et al, 2003; references therein). For terrestrial organic matters, the C:N ratios are normally over 12 (Savoye et al, 2003; references therein). Therefore, it is useful to classify phytoplankton from heterotrophs and terrestrial materials (Lobbes et al., 2000; Savoye et al., 2003; Lee and Whitledge, 2005). In this study, the mean C:N ratios of POM was 7.0 (S.D. $=\pm$ 0.4), which indicates that this POM is mainly phytoplankton (Table 3). However, the C:N ratio must be used with caution because of its variation in the process of POM degradation (Savoye et al, 2003). For example, PON is preferentially degraded compared to POC of phytoplankton, which causes an increase of the C:N ratio. Terrestrial organic matters (high C:N ratio) colonized by bacteria (low C:N ratio) lowers their initial high C:N ratio (Savoye et al, 2003; references therein). Therefore, similar C:N ratios of POM could be produced by degraded phytoplankton and bacteria-colonized terrestrial organic matters (Lancelot and Billen 1985; Savoye et al, 2003). In addition to C:N ratios, δ^{13} C of POM can be used for determining their origin. Kang et al. (2003) reported that the mean δ^{13} C signature of phytoplankton in Gwangyang Bay was -20.8 % (S.D. = \pm 1.1‰). In this study, our mean δ^{13} C signature of POM was -20.9 ‰ (S.D. = \pm 3.2‰), which also indicates that POM was mostly phytoplankton during the study periods (Table 3). Based on our C:N ratio and δ^{13} C value in this study, we confirmed that our POM samples were primarily comprised of phytoplankton in Gwangyang Bay.

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4. 3. Environmental conditions and biochemical pools

Biochemical pools of POM originating from phytoplankton are influenced by various environmental factors, such as temperature, salinity, nutrients, and light conditions (Morris et al., 1974; Smith and Morris, 1980; Rivkin and Voytek, 1987; Boëchat and Giani, 2008; Cuhel and Lean, 1987; Mock and Kroon, 2002; Khotimchenko and Yakoleva, 2005; Ventura et al., 2008; Sterner et al. 1997). In this study, significant relationships were found between environmental conditions and biochemical pools, especially PRT and LIP (Table 5). Temperature was positively and negatively correlated with LIP and PRT. Previous studies reported that higher temperature stress mainly affects nitrogen metabolism (Kakinuma et al., 2006) which is related to significant decrease of PRT with increases of LIP and CHO content (Tomaselli et al., 1988; Oliveira et al., 1999). In a high temperature-stressed condition of phytoplankton, the decrease in PRT content is related to breakdown of protein structure and interference with enzyme regulators (Pirt, 1975), whereas LIP is predominant because LIP is more closely associated with cell structure such as thickened cell walls (Smith et al., 1989; Kakinuma et al., 2001, 2006). Our results are in agreement with other works, as described above.

The relationships between nutrients and biochemical pools could be explained by nutrient limitation and the characteristics of each biochemical compound. A combination of nutrient concentrations and ratios can be used to assess nutrient limitation (Dortch and Whitledge, 1992; Justić et al., 1995). Dortch and Whitledge (1992) suggested that nutrient limitations are existed in the Mississippi river plume and Gulf of Mexico, if the dissolved inorganic phosphorus (DIP), dissolved inorganic nitrogen (DIN), and dissolved silicon (DSi) concentrations in water column are less than 0.2, 1.0 and 2.0 μM, respectively. In addition, molar ratios of the DIN:DIP and DSi:DIN can be indicators of nutritional status and the physiological behavior of phytoplankton (Redfield et al., 1963; Goldman et al., 1979; Elrifi and Turpin, 1985; Roelke et al. 1999). The following criteria of their molar ratios were (a) DSi:P ratio >16, and DSi:N ratio >16 for phosphorus (P) limitation; (b) DSi:DIP ratio >16 and DSi:N ratio >1 for nitrogen (N) limitation; (c) DSi:DIP ratio <16 and DSi:DIN ratio <1 for

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concentrations or/and their molar ratios depending on seasons (Table 6). Previous studies of biochemical composition in relation to nutrient limitation reported that PRT production of phytoplankton was enhanced under abundant N conditions (Fabiano et al., 1993; Lee et al., 2009). In contrast, LIP production and storage were dominant (Shifrin and Chisholm, 1981; Harrison et al., 1990) and PRT contents decreased (Kilham et al., 1997; Lynn et al., 2000; Heraud at al., 2005) under N-depleted conditions. High LIP contents have also been detected in phytoplankton under P or/and Si limitation (Lombardi and Wangersky, 1991; Lynn et al. 2000; Heraud et al., 2005; Sigee et al., 2007). Under N or P-limited conditions, triglyceride content (energy storage) increases and shifts from PRT to LIP metabolism since proteins are nitrogenous compounds whereas LIP and CHO are nonnitrogenous substrates (Lombardi and Wangersky, 1991; Smith et al., 1997; Takagi et al., 2000). In our study, Si and P concentrations may not significantly impact on biochemical composition of phytoplankton. Si concentrations were almost above 2.0 µM except in April 2013 during the study period. P limitation was observed based on the absolute concentration and molar ratios during study period. However, under P limitation, phytoplankton can relocate the cellular P pool to maintain their P requirements for the maximum growth rate (Cembella et al., 1984; Ji and Sherrell, 2008). In this respect, we suggest that DIN could be significantly impact on biochemical composition of phytoplankton in our study area. DIN was initially believed to be the most important limiting factor for phytoplankton growth in marine ecosystems (Ryther and Dunstan, 1971; Howarth, 1988). In fact, DIN was strongly positively correlated with PRT composition, whereas it was negatively correlated with LIP composition. The most of DIN loading came from freshwater input of the Seomjin River (Table 5, river-input vs NH₄ and NO₂+NO₃; r = 0.91 and 0.55, p < 0.01, respectively) influences on PRT and LIP synthesis and subsequently macromolecular composition of phytoplankton. As a result, the amount of river-input was also strongly correlated with PRT composition (Table 5 and Fig. 3). Therefore, DIN is an important controlling factor for biochemical composition, especially PRT and LIP composition of phytoplankton in Gwangyang bay.

silicate (Si) limitation. In this study, nutrient limitation conditions were observed by absolute nutrient

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Although irradiance is also known for an important governing factor for biochemical composition, irradiance was not significantly correlated with biochemical pools in this study (Table 5). We measured irradiance during our incubation time (4~5h) for phytoplankton productivity. This short time of measured irradiance might be not enough to reflect and detect the change of biochemical composition in phytoplankton with irradiance

The structure and composition of phytoplankton assemblages and species could have a significant influence on the seasonal variation of biochemical composition. Although we did not conduct a study of phytoplankton community structure, there is seasonal succession of phytoplankton community structure in the bay. Previous studies showed that the dominant phytoplankton community was diatoms and dominant diatom species were *Skeletonema spp.* during summer and winter in Gwangyang bay (Choi et al., 1998; Baek et al., 2015). Kim et al. (2009) also reported that diatom and dinoflagellate communities have experienced a considerable change because of increased nutrient loadings from both domestic sewage and industrial pollution during summer. Therefore, the seasonal change of phytoplankton species composition and community structure could lead to determining different biochemical pools on seasonal basis.

However, other studies in different regions reported that environmental conditions, such as temperature, nutrients and irradiance are more important controlling factors in biochemical composition than variation of phytoplankton community and species composition (Lindqvist and Lingnell, 1997; Suárez and Marañón, 2003). In this study, we also concluded that DIN from riverinput was a primary governing factor for the seasonal variation of biochemical composition of phytoplankton in Gwangyang Bay as discussed above.

4.4. Total FM and energy content of POM in a global context

The annual average of FM was 434.5 μ g L⁻¹ (S.D. = \pm 175.5 μ g L⁻¹) in this study. Since there were no comparable data available in South Korea, we compared our results with other regions (Table

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7), although they were conducted in different seasons and sampling depths. PRT contents in this study were as high as in the Ross Sea (Fabiano and Puscceddue, 1998; Fabiano et al., 1999a), the Amundsen Sea (Kim et al., 2015) and the Humboldt Current System (Isla et al., 2010). A similar range of LIP contents was observed in Bedford Basin (Mayzaud et al., 1989), Yaldad Bay (Navarro et al., 1993) and the Humboldt Current System (Isla et al., 2010). CHO contents were comparatively higher in this study than other studies except Bedford Basin (Mayzaud et al., 1989) and Yaldad Bay (Navarro et al., 1993). One of the highlights is that the calorific contents of FM were generally higher than those of other areas except several regions. The FM values were comparatively higher than other regions such as the northern Chuckchi Sea (Kim et al., 2014; Yun et al., 2014), Ross Sea (Fabiano et al., 1996; Fabiano and Pusceddu, 1998; Fabiano et al., 1999a; Pusceddu et al., 1999), Amundsen Sea (Kim et al., 2015) and the northern part of the East/Japan Sea (Kang et al., unpublished) or similar to the Humboldt Current System which is known as an important spawning sites for pelagic fishes and the highest abundance of anchovy eggs (Isla et al., 2010). Actually, the southern coastal sea (including our study area) in Korea represents calm seas, an indented coastline, and numerous bays, which have high diversities of habitat for fishes and shellfishes (Kwak et al., 2012) and give a favorable condition for mariculture (Kwon et al., 2004). The high quantity of FM and the calorific contents of POM found in this study reflected good nutritive conditions of primary food materials mainly provided by phytoplankton for the maintenance of productive shellfish and fish populations in Gwangyang bay.

5. Summary and Conclusion

This study is the first report that was investigated the biochemical composition of POM on seasonal basis in Gwangyang Bay, South Korea and we determined major controlling factors for biochemical composition which is influenced by various environmental factors (Morris et al., 1974; Smith and Morris, 1980; Rivkin and Voytek, 1987; Boëchat and Giani, 2008; Cuhel and Lean, 1987; Mock and Kroon, 2002; Khotimchenko and Yakoleva, 2005; Ventura et al., 2008; Sterner et al. 1997). Among different factors, temperature was positively correlated with LIP whereas negatively

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correlated with PRT in this study (Table 5), which is consistent with previous works. In addition, we found that PRT and LIP compositions were strongly correlated with DIN loading largely depending on the amount of river-input from the Seomjin river which influences on PRT and LIP synthesis and subsequently macromolecular composition of phytoplankton in Gwangyang bay. The concentrations and the calorific contents of FM found in this study were relatively higher in comparison to previous studies in various regions, which reflecting that POM (mainly from phytoplankton) provides a good nutritive condition to maintain this highly productive estuarine ecosystem in Gwangyang bay.

Recently, significant environmental perturbations in their watersheds and externally from climatic forcings have been reported in various estuaries (Wetz and Yoskowitz, 2013). More intense but less frequent tropical cyclones are expected over the coming century (e.g., Elsner et al., 2008; Knutson et al., 2010) and many changes in drought and flood cycles have been proceeding globally (e.g., Min et al., 2011; Pall et al., 2011; Trenberth and Fasullo, 2012; Trenberth, 2012). The cumulative effects of these perturbations could alter the quantity and quality of biochemical composition of POM and cause subsequent changes in ecosystem structure and trophic dynamics in estuaries (Cloern, 2001; Paerl et al., 2006; Rabalais et al., 2009; Wetz and Yoskowitz, 2013). Therefore, continuous field measurements and observations on biochemical composition of POM as food quality are needed to monitor—for better understanding future response of marine ecosystem on potential environmental perturbations in Gwangyang Bay.

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Table 1. Environmental factors and chl-a concentrations in Gwangyang Bay during the research period (-: no data).

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Year	Date	Irradiance $(\mu mols \ m^{-2} \ s^{-1})$	Station	Light depth (%)	Temperature (°C)	Salinity (‰)	Depth (m)	NH ₄ (μM)	${NO_2+NO_3 \atop (\mu M)}$	$SiO_{_{2}} \atop (\mu M)$	PO ₄ (μΜ)	Chl-a (μg L ⁻¹)
2012	April	167.9 ± 133.5	St.1	100	13.9	14.5	0	3.6	56.4	26.0	80.9	1.89
		(average \pm S.D.)		30	13.3	25.6	1	-	-	-	-	1.95
				1	13.5	28.0	3	2.4	16.0	9.8	0.2	2.08
			St.4	100	15.0	24.4	0	2.6	15.1	16.3	0.2	1.81
				30	13.6	31.4	1	-	-	-	-	-
				1	12.3	32.9	5	1.9	2.1	2.1	0.1	2.03
			St.5	100	12.6	31.7	0	3.1	9.5	7.1	0.3	2.07
				30	12.3	31.6	1	-	-	-	-	-
				1	12.2	32.4	5	3.0	6.4	5.1	0.3	2.04
	June	1158.1 ± 627.6	St.2A	100	22.9	27.6	0	-	-	-	-	1.77
				30	22.8	27.6	1	-	-	-	-	0.76
				1	22.9	28.7	3	-	-	-	-	0.76
			St.4	100	23.6	31.5	0	-	-	-	-	1.00
				30	22.6	31.9	3	-	-	-	-	1.67
				1	22.1	32.3	11	-	-	-	-	1.02
	August	1320.0 ±316.9	St.4	100	25.8	30.6	0	0.1	0.1	10.6	0.1	8.11
				30	25.7	31.6	2	-	-	-	-	8.49
				1	25.7	31.7	8	0.1	0.1	11.9	0.1	5.99
			St.5	100	25.6	31.6	0	0.7	0.3	8.2	0.0	14.20
				30	26.1	31.5	2	-		-	-	9.85
				1	25.7	31.7	8	0.1	0.1	10.1	0.1	3.19
	October	-	St.2A	100	20.6	29.8	0	1.4	3.0	11.3	0.1	1.07
				30	20.5	29.8	1	-	-	-	-	1.30
				1	21.9	30.2	3	1.3	1.3	8.1	0.1	1.24
			St.4	100	20.9	30.3	0	1.6	3.1	14.0	0.1	2.69
				30	20.7	30.3	1	-	-	-	-	2.93
				1	20.6	30.6	5	1.1	0.6	7.4	0.1	1.74
			St.5	100	19.1	30.4	0	1.0	0.4	6.5	0.1	2.47
				30	18.5	30.5	2	-	-	-	-	1.98
				1	18.1	30.4	8	1.2	0.2	5.3	0.0	2.20
					20							

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Table 1. (continued)

Year	Date	Irradiance (μmols m ⁻² s ⁻¹)	Station	Light depth (%)	Temperature (°C)	Salinity (‰)	Depth (m)	$\begin{array}{c} NH_4 \\ (\mu M) \end{array}$	$\begin{array}{c} NO_2 + NO_3 \\ (\mu M) \end{array}$	SiO ₂ (μM)	PO ₄ (μM)	Chl-a (μg L ⁻¹)
2013	January	297.4 ± 310.5	St.2A	100	5.5	20.5	0	0.5	4.2	4.0	0.1	1.3
				30	7.0	28.0	1	-	-	-	-	1.5
				1	7.3	29.4	4	0.5	3.7	3.6	0.1	1.4
			St.4	100	7.7	31.1	0	1.0	3.8	3.4	0.1	2.7
				30	7.4	31.3	4	-	-	-	-	3.
				1	7.3	32.8	12	0.6	3.1	2.5	0.0	5.
			St.5	100	6.3	31.8	0	0.8	3.3	2.6	0.1	5.
				30	6.6	31.9	3	-	-	-	-	5.
				1	6.4	32.5	11	1.0	3.0	3.6	0.2	5.
	April	1593.3 ± 414.5	St.2A	100	14.3	26.2	0	1.9	3.7	3.1	0.1	1.
				30	14.4	27.5	1	-	-	-	-	1.
				1	14.3	29.1	3	1.5	2.5	2.3	0.1	2.
			St.4	100	14.7	32.0	0	1.6	2.0	2.5	0.1	2.
				30	15.3	32.0	1	-	-	-	-	4.
				1	15.2	32.6	5	1.5	1.7	1.6	0.1	7.
			St.5	100	16.1	31.9	0	1.1	1.3	1.3	0.1	4.
				30	16.1	32.0	3	-	-	-	-	5.
				1	16.6	32.3	11	1.1	0.7	1.0	0.1	5.

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Table 2. Rainfall and river input

V	Data	Rainfall	River input
Year	Date	(mm)	(10 ⁶ t)
	April	195.5	149.4
	May	44.4	148.9
	June	69.6	42.3
	July	235.8	223.3
2012	August	559.0	228.9
	September	360.1	447.2
	October	38.0	98.5
	November	52.5	83.4
	December	96.7	89.4
	January	15.6	79.3
2013	February	116.4	94.6
2013	March	79.9	91.5
	April	99.1	100.3

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Table 3. δ¹³C values and C:N ratios of POM in Gwangyang Bay (surface)

Year	Date	δ ¹³ C (‰)	C:N (µg µg)				
2012	April	-22,8	7.0				
	June	-23.1	6.8				
	August	-16.5	6.7				
	October	-17.1	6.9				
2013	January	-22.5	7.7				
	April	-23.1	6.8				
(average ± S.D.)	-20.9 ± 3.2	7.0 ± 0.4				

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Table 4. Biochemical concentrations and composition, calorific values and contents in Gwangyang Bay (-: no data).

Year	Date	Station	Light depth (%)	CHO (μg L ⁻¹)	PRT (µg L ⁻¹)	LIP (µg L ⁻¹)	$FM \atop (\mu g \ L^{\cdot l})$	CHO/FM (%)	PRT/FM (%)	LIP/FM (%)	Kcal g ⁻¹	Kcal m ⁻³
2012	April	St.1	100	45.0	144.2	22.9	212.1	21.2	68.0	10.8	5.6	1.2
			30	53.1	218.6	51.9	323.6	16.4	67.6	16.0	5.9	1.9
			1	53.1	220.4	84.2	357.6	14.8	61.6	23.5	6.2	2.2
		St.4	100	14.2	128.1	28.6	170.9	8.3	74.9	16.7	6.1	1.0
			30	50.0	155.1	21.4	226.5	22.1	68.5	9.4	5.6	1.3
			1	20.2	146.0	37.3	203.5	9.9	71.8	18.3	6.1	1.2
		St.5	100	60.2	198.0	143.0	401.2	15.0	49.3	35.7	6.7	2.7
			30	132.4	198.0	42.8	373.2	35.5	53.1	11.5	5.5	2.0
			1	146.7	265.3	210.0	622.1	23.6	42.7	33.8	6.5	4.1
	June	St.2A	100	170.7	99.7	233.5	503.8	33.9	19.8	46.3	6.9	3.5
			30	135.5	108.0	251.9	495.4	27.3	21.8	50.9	7.2	3.5
			1	163.5	85.0	225.1	473.7	34.5	17.9	47.5	6.9	3.3
		St.4	100	99.1	44.6	199.5	343.2	28.9	13.0	58.1	7.4	2.5
			30	133.4	142.4	203.5	479.3	27.8	29.7	42.4	6.8	3.3
			1	91.6	110.8	232.3	434.6	21.1	25.5	53.5	7.3	
	August	St.4	100	69.3	73.9	213.5	356.7	19.4	20.7	59.9	7.6	
			30	61.2	56.5	173.8	291.5	21.0	19.4	59.6	7.6	2.2
			1	127.2	77.9	162.2	367.3	34.6	21.2	44.2	6.8	
		St.5	100	155.5	289.4	204.7	649.6	23.9	44.6	31.5	6.4	
			30	412.3	102.0	401.4	915.7	45.0	11.1	43.8	6.6	
			1	83.3	22.8	228.3	334.4	24.9	6.8	68.3	7.9	
	October	St.2A	100	71.0	82.2	104.1	257.3	27.6	32.0	40.5	6.7	
			30	42.7	62.4	100.3	205.4	20.8	30.4	48.8	7.2	
			1	74.3	111.6	98.5	284.4	26.1	39.2	34.6	6.5	
		St.4	100	51.6	105.2	105.3	262.2	19.7	40.1	40.2	6.8	
			30	119.4	121.9	144.4	385.6	31.0	31.6	37.4	6.6	
			1	78.5	169.0	134.4	381.9	20.6	44.2	35.2	6.6	
		St.5	100	37.2	70.0	86.5	193.6	19.2	36.1	44.7	7.0	
			30	42.3	92.5	112.0	246.7	17.2	37.5	45.4	7.1	1.7
			1	33.9	108.4	97.3	239.7	14.2	45.2	40.6	6.9	1.7

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Table 4. (continued)

Year	Date	Station	Light depth (%)	CHO (μg L ⁻¹)	PRT (μg L ⁻¹)	LIP (μg L ⁻¹)	$FM \atop (\mu g \stackrel{\cdot 1}{L})$	CHO/FM (%)	PRT/FM (%)	LIP/FM (%)	Kcal g ⁻¹	Kcal m ⁻³
2013	January	St.2A	100	150.3	139.3	115.5	405.2	37.1	34.4	28.5	6.1	2.5
			30	347.0	131.1	109.2	587.3	59.1	22.3	18.6	5.4	3.2
			1	331.3	127.1	-	-	-	-	-	-	
		St.4	100	171.6	164.0	-	-	-	-	-	-	
			30	183.5	168.7	139.7	491.9	37.3	34.3	28.4	6.1	3.0
			1	115.9	182.3	107.1	405.2	28.6	45.0	26.4	6.2	2
		St.5	100	113.6	212.0	133.4	459.0	24.7	46.2	29.1	6.3	2.9
			30	264.1	204.8	120.5	589.4	44.8	34.8	20.4	5.7	3.4
			1	99.3	195.5	104.2	399.0	24.9	49.0	26.1	6.2	2.5
	Apirl	St.2A	100	237.7	262.9	189.9	690.5	34.4	38.1	27.5	6.1	4.2
			30	185.5	308.0	198.7	692.3	26.8	44.5	28.7	6.3	4.3
			1	274.8	382.4	180.3	837.5	32.8	45.7	21.5	5.9	4.9
		St.4	100	115.0	141.9	181.4	438.4	26.2	32.4	41.4	6.8	3.0
			30	116.4	187.0	191.0	494.5	23.5	37.8	38.6	6.7	3
			1	205.2	222.1	185.7	612.9	33.5	36.2	30.3	6.2	3.
		St.5	100	160.4	176.3	289.1	625.7	25.6	28.2	46.2	7.0	4.
			30	146.9	217.8	253.3	618.0	23.8	35.2	41.0	6.8	4.3
			1	171.3	204.9	272.6	648.8	26.4	31.6	42.0	6.8	4.

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Table 5. Significant correlation coefficient (r) among proteins (PRT), lipids (LIP) and environmental factors (ns; no significance, **; p<0.01).

Variables	r	p	n
%PRT × Temp.	- 0.52	**	46
%LIP × Temp.	0.72	**	46
$\text{\%PRT} \times \text{NH}_4$	0.69	**	28
$\%$ LIP \times NH ₄	-0.59	**	28
$\text{\%PRT} \times \text{NO}_2 + \text{NO}_3$	0.54	**	28
$\%LIP \times NO_2 + NO_3$	-0.53	**	28
%PRT × River-input	0.84	**	46
%LIP × River-input	-0.63	**	46
NH₄ × River-input	0.91	**	28
$NO_2 + NO_3 \times River-input$	0.55	**	28
%PRT × %LIP	-0.81	**	46
%PRT × Irradiance	-0.22	ns	39
%LIP × Irradiance	0.24	ns	39

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Table 6. Observed nutrient limitations during the study period.

	Date	Based on ab	solute concent	rations (μM)	Based on molar ratios				
Year		DIN	SiO ₂	PO ₄	Limitation	Si:N	Si:P	Limitation	
2012	April	20.3 ± 20.2	11.1 ± 8.8	13.6 ± 32.9	nd	0.6 ± 0.2	37.5 ± 36.9	nd	
	June	-	-	-	-	-	-	-	
	August	0.4 ± 0.4	10.2 ± 1.5	$\textbf{0.1} \pm \textbf{0.0}$	N, P	42.7 ± 23.7	173.4 ± 56.5	N, P	
	October	2.7 ± 1.5	8.8 ± 3.3	0.1 ± 0.0	P	3.6 ± 0.8	142.2 ± 74.0	N	
2013	January	4.2 ± 0.4	3.3 ± 0.6	0.1 ± 0.1	P	$\textbf{0.8} \pm \textbf{0.1}$	50.6 ± 41.4	nd	
	April	3.4 ± 1.3	2.0 ± 0.8	0.1 ± 0.0	P	0.6 ± 0.1	15.5 ± 5.5	Si	

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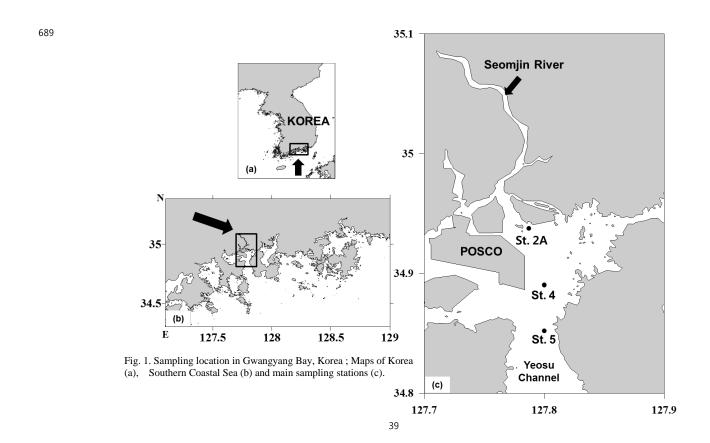


Table 6. Comparison of biochemical quantity of POM, FM and the calorific contents.

Regions (depth)		PRT (μg l ⁻¹)	LIP (μg l ⁻¹)	CHO (μg l ⁻¹)	FM (μg l ⁻¹)	Kcal m ⁻³ (average ± S.D.)	Authors
	Gwangyang Bay, South Korea (Euphotic depth)	23-382	21-401	14-412	171-916	2.8 ± 1.1	This study
Arctic regions	Bedford Basin, Canada(2.5 m)	200-650	130-440	160-630	660-1570		Mayzaud et al. (1989)
	Logy Bay, Newfoundland (6 m)	80-740	20-75	8-120	130-1030	2.7 ± 2.8	Navarro & Thompson (1995)
	The Northern Chukchi Sea, 2011 (Euphotic depth)	1-86	50-105	22-147	94-246	1.0 ± 0.2	Kim et al. (2015)
	The Northern Chukchi Sea, 2012 (Euphotic depth)	9-183	37-147	16-253	90-373	1.2 ± 0.2	Yun et al. (2015)
Antarctic regions	Pacific Sector Antarctic Ocean (0-1500 m)	14-100	3-60	3-66	25-220		Tanoue (1985)
	Off Princess Astrid Coast, Antarctica (0-100m)	24-200	15-174	22-147	148-393		Dhargalkar et al. (1996)
	Ross Sea, Antarctica (10m)	11-402	91	91-187	193-680	$\pmb{2.6 \pm 1.8}$	Fabiano and Pusceddu (1998)
	Ross Sea, Antarctica (0-200 m)	40-406	18-115	22-251	110-660		Fabiano et al. (1999)
	Terra Nova Bay, Antarctica (0-750 m)	10-620	2-77	8-144	19-885	1.3 ± 1.0	Fabiano et al. (1996)
	Terra Nova Bay, Antarctica (under pack ice)	96-201	38-112	10-68	145-382	1.7 ± 1.1	Pusceddue et al. (1999)
	Amundsen Sea (Euphotic depth)	6-396	13-37	3-216	43-639	1.2 ± 0.8	Kim et al. (2015)
Other regions	W-Mediterranean (0-200 m)	72-105	37-51	33-88	143-246		Fabiano et al. (1984)
	W-Mediterranean submarine cave (10m)	4-77	4-104	1-75	15-220	0.4 ± 0.2	Fichez (1991b)
	Mediterranean seagrass (4 m)	25-135	50-180	40-110	125-395		Danovaro et al. (1998)
	Ligurian Sea (10 m) NW-Mediterranean	32-107	21-140	21-131	74-378	1.5 ± 1.4	Danovaro & Fabiano (1997)
	Mediterranean (30m)	70-90	90-110	10-20	177-213	1.4 ± 0.2	Modica et al. (2006)
	Cretan Sea (0-1500 m)	7-92	4-63	13-149	54-200	0.6 ± 0.2	Danovaro et al. (2000)
	Bay of Biscay, 2000 (0-30m)	109-2426	26-2037	2-345	961 (a.v.)	6.7 ± 5.0	Díaz et al. (2007)
	Yaldad Bay, Chile (10 cm a.b.)	300-2250	30-560	50-1050	3310-2960	10.0 ± 10.9	Navarro et al. (1993)
	The Humboldt current system, Northern Chile (5-89m)	40-470	60-390	70-510	24-1282	3.5 ± 3.3	Isla et al. (2010)
	Magellan Strait (0-50m)	60-150	30-70	20-40	110-256	1.0 ± 0.5	Fabiano et al. (1999)
	The northern part of the East Sea (Euphotic depth)	28-425	12-180	19-206	109-810	1.5 ± 0.6	Kang et al. (unpublished)







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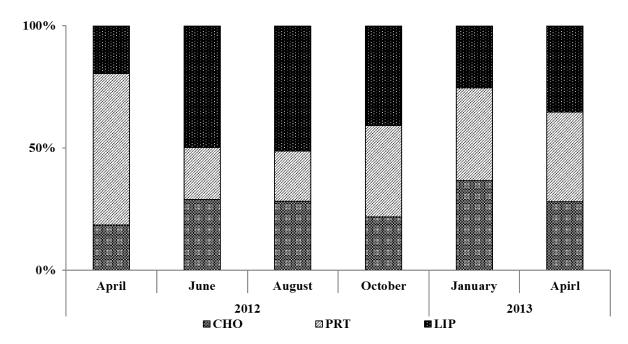


Fig. 2. Seasonal variation of biochemical composition in Gwangyang Bay.

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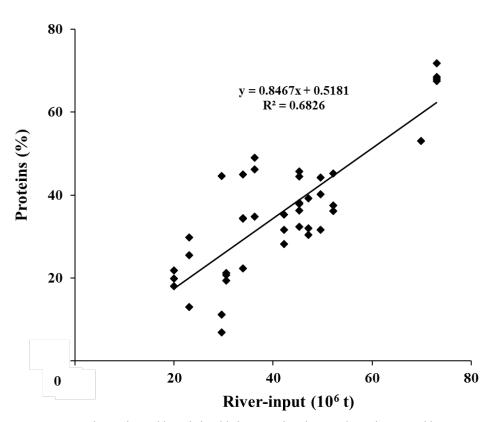


Fig. 3. The positive relationship between river-input and protein composition.

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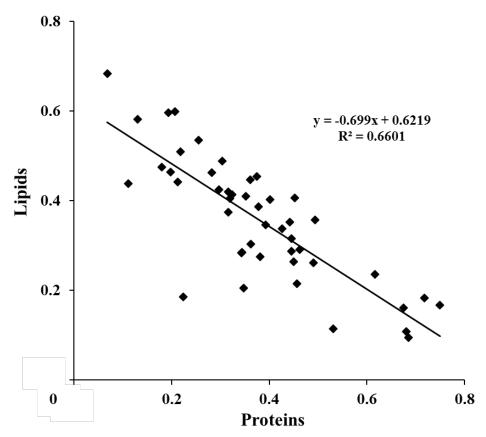


Fig. 4. The inverse relationship between lipid compositions and protein compositions