

1 **Interactive comment on “The effects of different environmental factors**
2 **on biochemical composition of particulate organic matters in**
3 **Gwangyang Bay, South Korea” by Jang Han Lee et al.**

4
5 **Anonymous Referee #1**

6
7 Received and published: 16 September 2016

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9 Review of Biogeosciences Discuss., doi:10.5194/bg-2016-347 The effects of different environmental
10 factors on biochemical composition of particulate organic matter in Gwangyang Bay, South Korea
11 written by Jang Han Lee, Dabin Lee, Jae Joong Kang, Hui Tae Joo, Jae Hyung Lee, Ho Won Lee, So
12 Hyun Ahn and Sang Heon Lee In the submitted article the authors analyzed seasonal changes of the
13 biochemical composition (proteins, lipids, carbohydrates) of the particulate organic matter and linked
14 it to environmental factors in order to determine the major environmental factor influencing the
15 changes of biochemical composition and the origin of particulate organic carbon.

16 In general, the paper has a scientific potential and some parts of the paper are fairly discussed
17 (biochemical composition) and linked to the relevant literature. However, some parts of the sections
18 Materials and methods, Results and Discussion are not clearly outlined or missed important
19 information that complicate understanding of the text and question the purpose of applied
20 experimental design. The conclusions are mostly repeating of the results so it should be also rewritten
21 and the last paragraph omitted, it is too general and does not contain the conclusion of the paper. The
22 major revision and resubmission is recommended.

23 →We revised each section throughout the manuscript, deleted most of repeating results in
24 conclusions and revised carefully our manuscript based on referee # 1 comments as below.

25
26 The experimental design was based on three different light intensity depths along three stations in bay
27 and all results were pooled together on the monthly basis since no significant differences between
28 vertical and spatial distributions were found. It was mentioned in the Material and methods that some
29 statistical tests (ANOVA, t-test) were used, but it is not clear which test they used, where and which
30 parameters they tested and how (there is 1 concentration per 1 depth at 1 station).

31 → We did ANOVA test for each depth from 3 stations based on an assumption of no spatial
32 difference and another ANOVA test for a spatial difference by pooling of 3 light depths at one station
33 and comparing each station based on an assumption of no difference in light depths. But, we found to
34 realize that there are statistical errors by doing that. So, we deleted no significant differences between
35 vertical and spatial distributions.

36
37 The authors used very often describing results the word significant but did not specified the name of
38 test, F-value or t-value.

39 →We revised our results.

40
41 Details and reference about determination and/or calculation of the 30% and 1% of the photon flux
42 based on Secchi disc depths should be added.

43 →We added details and reference for light depth determination in line 87-90, page 4-5.

44
45 It was only mentioned that the samples were incubated and later on in discussion it was written that
46 the incubation time was too short?

47 →Our main purpose of the PAR measurements was calculating hourly primary productivity executed
48 for 4~5 hours as a parallel study. Therefore, the irradiance values measured in this study were not
49 representative for our sampling periods. We mentioned this in the method section in line 92-97, page 5
50 and further discussed on the issue in line 370-379, page 16.

52 Further on, the light intensity and its impact (or no impact) on the biochemical composition is not
53 discussed, particularly considering 10 times difference in light intensity between April 2012 and April
54 2013. These findings should be discussed with regard to a body of literature in which the influence of
55 light was investigated and found.

56 →We added the discussion on the light intensity impact on the biochemical compositions, especially
57 10 times difference in light intensity between April 2012 and April 2013 in line 370-379, page 16.

58
59 In the Table 1 there is irradiance expressed as $\text{ave} \pm \text{S.D.}$; I wonder if given average contains
60 measurements from all stations on the day of sampling?

61 →We measured irradiance one time per each cruise at every 30 seconds during the incubation hours
62 for primary productivity executed for 4~5 hours during day time around local noon time. So, $\text{ave} \pm \text{S.D.}$
63 values in Table 1 are averages from every 30 seconds for 4-5 hours a day each season. We described
64 the details in line 95-97, page 5.

65
66 Details about particulate organic carbon and nitrogen analysis such as volume of filtered water and
67 station where the sample was taken should be added (only one result per month was presented). This
68 is very important since the origin of POM is not typical for the estuaries.

69 →We measured POC, PON, and $\delta^{13}\text{C}$ of POM collected from surface at the 3 stations at every
70 sampling time. They varied but did not show large differences in POC, PON, and $\delta^{13}\text{C}$ among the
71 different stations. We described the sampling details in line 114-120, page 6.

72
73 It is very interesting that riverine terrestrially derived organic matter is not an important component of
74 the particulate organic matter in the Gwangyang Bay system, which has a large river runoff. One
75 would expect partly organic matter of a terrestrial origin and not such clear phytoplankton fingerprint
76 since the water column is very turbid and euphotic layer very thin (3- 11 m). Also this peculiarity and
77 these results should be discussed and compared with other estuaries like the authors did for
78 biochemical composition.

79 →We further discussed on the issue in line 300-310, page 13-14.

80
81 Nutrient limitation, the use of the ratios (lines 301-305): it is not clear why the authors use for the
82 interpretation of phosphorus and nitrogen limitation only the ratios with dissolved silica (DSi) and not
83 between these two components (N: P). If it was not a random error, the reference should be added for
84 listed criteria. Anyway, in criteria b) for nitrogen limitation instead of DSi:DIP ratio >16 should stand
85 < 16 , if it was presumed that DSi and DIN appear in similar concentrations, though not always the
86 case.

87 →We revised them in line 335-338, page 15 based on Dortch and Whitley (1992).

88
89 References: Listed but not cited in the text: Adolf and Harding, 2006; Choi and Noh, 1998; De
90 Oliveira et al 1999; Julian and David, 1966; Kim et al., 2016 Cited in the text but not listed: Choi et
91 al., 1998; Kim et al., 2014; Kwon et al, 2001; Marsh and Weinstein 1966; Paerl et al.,
92 2006; Yun et al., 2014 Cited or listed with different year of publication: Pirt 1975 (cited in the text),
93 listed in references as Pirt 1976 Some references are written in uppercase. To the references published
94 in the same year a, b should be added

95 →We revised the references.

96
97 In Tables 1 and 4 in April 2012 appears st. 1 which is not marked on the map (Fig.1)

98 →We revised the map in Fig. 1.

99

100 **Interactive comment on “The effects of different**
101 **environmental factors on biochemical**
102 **composition of particulate organic matters in**
103 **Gwangyang Bay, South Korea” by Jang Han Lee et**
104 **al.**

105 **Dr. Yun** (misunyun@pusan.ac.kr)
106 Received and published: 27 September 2016

107
108 General Comments The manuscript presents the seasonal variation of biochemical composition of
109 POM in Bay. The author shows the major controlling factor for them based on statistical analysis.
110 Overall, I found the paper to be sound and believe that it contains valuable data in understanding the
111 characteristics of POM and their contribution to coastal ecosystem as basic food source. I think that
112 the paper is worthy of publication for BGS after minor revisions are made, while there are a few areas
113 that need improvement.

114
115 Major comment and corrections

116 1. Page 12, Line 258-278: The author showed $\delta^{13}C$ value and carbon to nitrogen ratio in surface, in
117 order to find the origin of POM. I think that the contribution of benthic microalgae to POM could be
118 large and significant, since the study area is located in coastal area and extremely turbid condition
119 related to freshwater input or tidal cycles or wind. Therefore, many amounts of benthic microalgae
120 could be included to POM through the resuspension, especially during high river input. Indeed, Table
121 3 shows the lower $\delta^{13}C$ value in August.

122 →We discussed on potential contributions of benthic microalgae on POM in line 300-302, page 13.

123
124 2. Pages 13-14, Line 301-304: For the criteria of their molar ratios among dissolved inorganic
125 nutrients, I wonder could it be applied in coastal area. I think that the status of nutrient limitation in
126 phytoplankton could be different between open oceans and coastal area.

127 →Actually, the criteria of the molar ratios can be applied in coastal area based on several papers as
128 we referred in our discussion (e.g., Roelke et al., 1999).

129
130 3. Page 15, Line 335-344: As the author discussed, I think that the composition of phytoplankton
131 assemblages and species could be closely related to seasonal variation of biochemical composition.
132 High nitrogen supply during river-input increased season could lead to different phytoplankton
133 composition. For example, the large sized phytoplankton (such as diatom) could be thrived in that
134 condition, since the large phytoplankton could grow best and dominate under eutrophic condition.
135 According to Fernandez et al. (1994), the carbon allocation into different biochemical pools were
136 different depending on dominant phytoplankton group. For example, the carbon allocation into lipids
137 was higher under the dominance of flagellates, whereas the lower lipid synthesis was observed in the
138 dominance of diatoms. Therefore, the seasonally different phytoplankton composition related to
139 nutrient input could affect to the different biochemical composition in the region.

140 →Yes, the seasonal compositions of phytoplankton could lead different biochemical compositions.
141 We discussed on that issues in line 380-389, page 15-16.

142
143 4. In figure 3, the author shows positive relationship between river input and protein composition.
144 However, I didn't find the positive relationship between them, based on comparison with table 2 and
145 figure 2. For example, the protein composition in August was lowest, although the river input was
146 considerably high. In addition, the protein composition from October in 2012 to April in 2013 was
147 higher than that in August, even though the lower river inputs were recorded.

148 →Actually, the river input data in Table 2 are monthly integrated river inputs to show monthly
149 patterns of river input and rainfall. In Figure 3, river-inputs were integrated from 20 days prior to our
150 sampling dates since phytoplankton productivity is recovered after 20 days after rainfall in
151 Gwangyang Bay according to Min et al. (2011). We added this explanation in line 100-102, page 5 to
152 make it clear.

153
154 Minor corrections

155 1. Pages 8-9, Line 175-186: The position of some sentences needs to be corrected. For example, the
156 results about irradiance and chl-a are shown in Table 1 (it is explained in line 178-186). The results
157 for rainfall and river-input are indicated in former position (in line 175-178), although they are shown
158 in Table 2.

159 →We rearranged the sentences in line 190-194, page 9.

160 2. Page 9, Line 195-197: The author found that there were no significant differences in spatial
161 distribution of POM. However, the protein composition in station 2A (is closest to the River) might be
162 higher than in station 4 and 5, since there is the large effect of river-input on the biochemical
163 composition in this study.

164 →We did ANOVA test for each depth from 3 stations based on an assumption of no spatial difference
165 and another ANOVA test for a spatial difference by pooling of 3 light depths at one station and
166 comparing each station based on an assumption of no difference in light depths. But, we found to
167 realize that there are statistical errors by doing that. So, we deleted no significant differences between
168 vertical and spatial distributions in our text. The station 2A (is closest to the River) might be the
169 largest effect of river-input but different effects of river-input could be different depending on water
170 circulation, tidal currents, winds, and etc as well as distance from the river in Gwangyang Bay. At this
171 point, we can not determine how much effect at each station from river inputs but the station 2A could
172 have more proteins than others if they have more influence from river inputs based on Table 1 and Fig.
173 3.

174

175 **Interactive comment on “The effects of different**
176 **environmental factors on biochemical composition of**
177 **particulate organic matters in Gwangyang Bay, South Korea”**
178 **by Jang Han Lee et al.**

179
180 **Anonymous Referee #2**

181 Received and published: 30 January 2017

182
183 The manuscript, “The effects of different environmental factors on biochemical composition of
184 particulate organic matter in Gwangyang Bay, South Korea” written by Lee et al., describes the
185 seasonal changes of the biochemical composition such as proteins, lipids, and carbohydrates of the
186 particulate organic matter and investigates the major environmental controlling factors for the changes
187 of biochemical composition. This paper is very interesting to present seasonal biochemical
188 composition of particulate organic matter responding to various environmental factors and the food
189 quantity assessed in Gwangyang Bay since previous studies have focused mainly on different
190 biochemical compositions as the authors mentioned that. The present study has scientific merits and
191 originality in that: 1. the topic, “biochemical composition of particulate organic matter as indicators of
192 food quality and quantity”, is very intriguing enough to draw much attention for understanding marine
193 ecosystem especially here in Gwangyang Bay; 2. the study is one of few studies that employed
194 seasonal biochemical composition of particulate organic matter responding to various environmental
195 factors; 3. authors found that river-derived dissolved inorganic nitrogen loading is a major governing
196 factor for determining the biochemical composition in a natural bay system which implies that man-
197 made artificial dams could cause a serious disturbance in the ecosystem. Overall, I would recommend
198 publication of this manuscript for Biogeosciences after some minor revisions. I hope to see authors
199 undertake revisions in an appropriate manner because I really want to see the final version of this
200 paper in print.

201
202 Some minor comments are listed below:

203 -. Be consistent with Gwangyang Bay or bay throughout the text.

204 → We checked it throughout the text.

205 -. Be consistent with average or mean in the results.

206 → We checked it throughout the text.

207 -. (Section 2.1) What time did usually authors make samplings? I’m wondering if there is tidal
208 influence and/or diurnal changes?

209 → There might be tidal influence (no diurnal changes) so that we sampled waters at high tide period
210 before the noon to reduce the tidal effects. We added sampling times in line 88-89, page 4-5.

211 -. (line 76) indicate t (ton ?).

212 → Yes, it is ton! We changed t into ton in line 78, page 4.

213 -. (lines 83-83) Describe how to determine 3 light intensities from secchi disk

214 → We described how to determine 3 light depths from secchi disk in line 89-91, page 5.

215 -. (line 157 in section 2.5) Describe what Winberg (1971) is.

216 → We described the Winberg equation in line 173, page 8.

217 -. (3. Results) Indicate statistics results in the result section.

218 → We indicated statistics results.

219 -. (Section 4.1) The first sentence in 4.1 section is repeated as the result section of 3.1. Remove it.

220 → We rephrased it.

221 -. (Table 6) No description for Table 6 is shown in the result section. Describe it in the result section
222 before the discussion on that in the discussion section.

223 → We changed Table 6 into Table 2 and described the result for the table.
224 -. (Section 4.4) The first sentence in 4.4 belongs to the result.
225 → We removed the sentence.
226 -. (Table 7) Be consistent with the unit in Table 7 and the text.
227 → We changed the unit in Table 7 in consistency of the text.
228 -. The conclusion section is needed to be revised since some parts in the conclusion repeat some
229 results.
230 → We removed the conclusion section because it is too general and does not contain the conclusion of
231 the paper.
232
233 |
234

235 **The effects of different environmental factors on biochemical composition of particulate**
236 **organic matters in Gwangyang Bay, South Korea**

237

238

239 **Jang Han Lee¹, Dabin Lee¹, Jae Joong Kang¹, Hui Tae Joo¹, Jae Hyung Lee¹, Ho Won**

240 **Lee¹, So Hyun Ahn¹ and Chang Keun Kang², Sang Heon Lee¹**

서식 있음: 위 첨자

241

242 ¹Department of Oceanography, Pusan National University, Geumjeong-gu, Busan 46241,

243 Korea

244 ²School of Environmental Science and Engineering, Gwangju Institute of Science and Techn

서식 있음: 글꼴: (영어) Times New Roman

245 ology, Gwangju 500-712, Korea

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248 *Corresponding author: sanglee@pusan.ac.kr

249

250 **Abstract**

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

271

272 **Key words:**

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

274 **1. Introduction**

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

287 The coastal areas represent one of the world's most vital aquatic resources, supporting and
288 providing food resources and habitats for large numbers of fish and shellfish species (Kwak et al.,
289 2012; Wetz and Yoskowitz, 2013; references therein). In Gwangyang Bay, the southern coast of
290 Korea (Fig. 1), coastal fisheries and shellfish farming have been prevalence. Over the past decades,
291 the bay have become industrialized such as the construction of steel mill company, power plant and
292 industrial complex and environmental disturbances have been predicted. Also, estuaries have a high
293 short-term variability depending on many episodic events, such as freshwater inputs, tidal cycles
294 (neap-spring), and wind (storms) (Cloern and Nichols, 1985). These anthropogenic forces and
295 environmental changes drastically affect the estuarine habitat properties which can cause different
296 biochemical compositions of POM. Unfortunately, little information is yet available on the
297 biochemical composition of POM in the bay, South Korea. Hence, this study tested the question of the
298 main environmental factors determining seasonal variation and of biochemical composition POM and

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

306 **2. Materials and methods**

307 **2.1. Study site and sampling procedure**

308 The study site was located in Gwangyang Bay (34.9 ° N, 127.8 ° E), the southern coast of
309 Korea (Fig. 1). The total area of the bay is 230 km² at mean sea level (Kang et al., 2003). The bay is
310 characterized by semidiurnal tides with a maximal range of about 4.8 m at spring tides (Korea
311 Hydrographic and Oceanographic Administration). Freshwater flows into the bay from the Seomjin
312 River at the northern part of the bay (mean flow 27 m³ s⁻¹ and annually 1.9 × 10⁹ [ton](#) during the
313 study period; the National Institute of Environmental Research) and seawater enters through the
314 narrow southern channel (Yeosu Channel).

315 To obtain data for seasonal variation of POM in the euphotic depth, the field samplings were
316 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,
317 June, August, and October in 2012 and January and April in 2013. St. 1 was changed to St. 2A after
318 April 2012 because of logistic problems. Both stations have similar environmental conditions at a
319 relatively close distance. Using a 5 L Niskin water sampler, water samples were collected at different
320 depths of 3 light intensities (100%, 30%, and 1% of surface irradiances; hereafter 3 light depths)
321 ~~which were determined by a secchi disk~~ and transferred to brown sample bottles which were
322 previously washed with a solution of 0.1 N HCl. [The water samplings were conducted at high tide](#)

323 periods before the noon. The different 3 light depths were determined by a secchi disk using vertical
324 attenuation coefficient ($K_d = 1.7/\text{secchi depth}$) from Poole and Atkins (1929) which have been applied
325 globally.

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서식 있음: 아래 첨자

326 To obtain *in situ* physical parameters, water temperature and salinity were measured with
327 YSI-30 (YSI incorporated) and photosynthetically active radiation (PAR) was measured onboard
328 during the cruise. PAR was measured one time per each cruise at every 30 seconds during the
329 incubation hours for primary productivity by a quantum sensor (LI-190SA, LI-COR) with a data
330 logger (LI-1400, LI-COR) on deck. Since the main purpose of the PAR measurements was calculating
331 hourly primary productivity executed for 4~5 hours during day time around local noon time, the
332 irradiance values in this study might be not representative for our sampling periods. Rainfall and
333 river-input data during the study period were obtained from the Korea Meteorological Administration
334 (<http://www.kma.go.kr/index.jsp>) and the National Institute of Environmental Research
335 (<http://water.nier.go.kr/main/mainContent.do>). For relationships between river-input and other factors,
336 river-inputs were integrated from 20 days prior to our sampling dates since phytoplankton
337 productivity is recovered after 20 days after rainfall in Gwangyang Bay according to Min et al. (2011).

338 **2.2. Chl-*a* and major inorganic nutrient analysis**

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

347 laboratory in the East Sea Fisheries Research Institute (QUAATRO, Seal Analytical).

348 2.3. Particulate organic carbon and nitrogen analysis

349 The water samples (300 ml) for POC, PON, and $\delta^{13}\text{C}$ of POM were collected from surface at
350 the 3 stations at every sampling time. The water samples were filtered through pre-combusted (450 °C,
351 4 h) 25 mm GF/F (Whatman, 0.7 μm) using a low vacuum pressure less than 5 in. Hg. The filters for
352 POC, PON, and $\delta^{13}\text{C}$ values were preserved frozen (-20 °C) for further analysis at home laboratory.
353 For stable isotope analysis, the preserved filters were acidified by concentrated hydrochloric acid
354 fumes overnight to remove carbonate (Hama et al., 1983) and the abundances of ^{13}C and ^{15}N and
355 the total amounts of POC and PON were determined using a Thermo Finnigan Delta + XP mass
356 spectrometer at the stable isotope laboratory of the University of Alaska Fairbanks, USA.

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357 2.4. Biochemical composition analysis

358 The water samples for the biochemical composition (carbohydrates, proteins, and lipids) of
359 POM were collected from 3 light depths. The water samples were filtered through 47 mm GF/F
360 (Whatman, 0.7 μm pore), which were immediately frozen at -70 °C and preserved for biochemical
361 composition analysis at the home laboratory.

362 *Protein analysis*

363 Protein (PRT) concentrations were assessed according to a modified method of Lowry et al.
364 (1951). The filters for PRT analysis were transferred into 12 mL centrifuge tubes with 1 mL DH_2O ,
365 respectively. The filters were grounded (using a glass rod) in the tubes with a 5 ml alkaline copper
366 solution (a mixture of 2% Na_2CO_3 in 0.1 N NaOH with 0.5% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 1 % sodium or
367 potassium tartrate; 50:1, v/v). The solutions for PRT concentrations were mixed well (using a vortex)
368 and allowed to stand for 10 min at room temperature in the hood. After 10 min, 0.5 mL of diluted
369 Folin-Ciocalteu phenol reagent (1:1, v/v) was added into the solution, mixed occasionally with a

370 vortex mixer, and allowed to sit for 1 h 30 min. The solutions with a blue color were centrifuged at
371 3,000 rpm for 10 min. Absorbance of the supernatant was measured at 750 nm. Bovine Serum
372 Albumin (2 mg mL⁻¹, SIGMA) was used as a standard for the PRT concentration.

373 *Lipid analysis*

374 Lipid (LIP) concentrations were extracted according to a column method modified from
375 Bligh and Dyer (1959), and Marsh and Weinstein (1966). The filters for LIP analysis were transferred
376 into 16 mL glass tubes with 3 mL of chloroform-methanol (1:2, v/v). The filters in the tubes were
377 grounded, and then the mixtures were mixed using a vortex mixer. For LIP extraction, glass tubes
378 with samples were stored in the fridge (4 °C) to prevent the solvents from evaporating. After 1 h, the
379 solvents were centrifuged at 2,000 rpm for 10 min and the supernatants were collected and stored in
380 new tubes. This extraction procedure was performed once again immediately. When the extractions
381 were completed, 4 mL of DH₂O was added to the solution in the new tubes, and the solution was
382 homogenized using a vortex mixer. After mixing, the tubes were centrifuged at 2,000 rpm for 10 min,
383 and the solvents were separated into two phases (the chloroform phase for lipids and methanol +
384 DH₂O phase). The methanol + DH₂O phase was removed from the solvent using a Pasteur pipette.
385 The chloroform phase was placed in a dry oven at 40 °C for 48 h. After it totally dried for
386 carbonization analysis (Marsh and Weinstein 1966), 2 mL of H₂SO₄ was added to the tubes and they
387 were placed in a heating block at 200 °C for 15 min. After this heating procedure, the tubes were
388 quickly placed in a water bath at room temperature; 3 ml of DH₂O was added to the tubes and the
389 solvents were homogenized (with a vortex mixer) and stood for 10 min or until all bubbles had
390 disappeared. Absorbance of the supernatant was measured at 375 nm. Tripalmitin solutions were used
391 as a standard for the LIP concentration.

392 *Carbohydrate analysis*

393 Carbohydrate (CHO) concentrations were measured according to Dubois et al. (1956). The

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

402 2.5. Statistical analyses and calorific value calculation

403 Statistical tests were carried out using the statistic software “SPSS” (*t*-test, ANOVA and Pearson’s
404 Correlation Coefficient). The level of significance was set at $p < 0.05$. The calorific value (Kcal g⁻¹) of
405 the food material (FM) (FM was defined by Danovaro et al. (2000); PRT + LIP + CHO concentrations;
406 hereafter FM) and the calorific content of FM (Kcal m⁻³ = Kcal g⁻¹ × g FM m⁻³) were calculated using
407 the Winberg (1971) equation ($\text{Kcal g}^{-1} = 0.055\% \text{ Proteins} + 0.041\% \text{ Carbohydrates} + 0.095\% \text{ Lipids}$).

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408 3. Results

409 3.1. Seasonal distribution and variation of environmental factors and chl-*a* concentrations

410 The values of environmental factors were summarized in Table 1. The temperature ranged
411 from 5.5 °C to 26.1 °C. ~~The temperature increased from April to August (the highest temperature in~~
412 ~~August 2012 at St. 4: 26.1 °C) and decreased from August to January (the lowest temperature in~~
413 ~~January 2013 at St. 2A: 5.5 °C). T~~and the salinity ranged from 14.5 ‰ to 32.9 ‰ ~~during our sampling~~
414 ~~period. Generally, the salinity increased from St. 1 or St. 2A to St. 5.~~ Relatively lower salinity, which
415 is mainly affected by fresh water input from the Seomjin River, was observed at St. 2A. The annual
416 average euphotic depth was 6.5 ± 3.4 m, ranging from 2 m to 12 m.

417 The highest nutrient concentrations were measured in April 2012, when the concentrations of
418 $\text{NO}_2 + \text{NO}_3$, SiO_2 , NH_4 , and PO_4 were above 5.0 μM , 2.0 μM , and 0.2 μM , respectively, except at 1%
419 light depth at St. 4. All inorganic nutrients except SiO_2 were nearly depleted in August 2012 (Table 1).
420 During the rest of our study period, $\text{NO}_2 + \text{NO}_3$ and SiO_2 concentrations were observed with similar
421 decreasing patterns from St.1 or St. 2A to St. 5. NH_4 concentrations averaged from October 2012 to
422 April 2013 were $1.1 \mu\text{M} \pm 0.4 \mu\text{M}$, ranging from 0.5 μM to 1.9 μM . PO_4 concentrations (average \pm
423 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~~
424 ~~St. 2A in April 2012~~ during the study period. For determining the nutrient conditions, nutrient
425 concentrations and their molar ratios in this study were summarized in Table 2. The ranges of the
426 molar ratios from April, 2012 to April, 2013 were 9.8-69.5, 15.5-173.4, and 0.6-42.7 for DIN:DIP,
427 DSi:DIP and DSi:DIN, respectively (Table 2).

428 Surface irradiance averaged from each measurement for 4-5 hours ranged from $167.9 \pm$
429 133.5 to $1593.3 \pm 414.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ (average \pm S.D.) from April 2012 to April 2013. The highest
430 and lowest irradiance were recorded in April 2013 and April 2012, respectively. Chl-*a* concentrations
431 in the euphotic depth ranged from $0.8 \mu\text{g L}^{-1}$ to $14.2 \mu\text{g L}^{-1}$ during the study period (annual
432 meanaverage \pm S.D. = $3.4 \mu\text{g L}^{-1} \pm 2.8 \mu\text{g L}^{-1}$; Table 1).

433 Monthly rainfall and river-input in the study location ranged from 15.6 mm to 559.0 mm
434 (annual meanaverage \pm S.D. = $151.0 \text{ mm} \pm 155.5 \text{ mm}$) and 42.3 to $447.2 \times 10^6 \text{ t}$ (annual meanaverage
435 = $144.4 \times 10^6 \text{ t}$), respectively (Table 23). Rainfall and river-input were recorded as high during
436 summer and low during winter (Table 2). ~~Average~~

437 ~~irradiance during our incubation hour ranged from 167.9 ± 133.5 to $1593.3 \pm 414.5 \mu\text{mol m}^{-2} \text{s}^{-1}$~~
438 ~~(average \pm S.D.) from April 2012 to April 2013. The highest and lowest irradiance were recorded~~
439 ~~in April 2013 and April 2012, respectively.~~

440 ~~Chl-*a* concentrations in the euphotic depth ranged from $0.8 \mu\text{g L}^{-1}$ to $14.2 \mu\text{g L}^{-1}$ during the~~

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

446 3.2. $\delta^{13}\text{C}$ values and carbon to nitrogen ratios of POM

447 $\delta^{13}\text{C}$ values of sea surface POM ranged from - 23.1 ‰ to - 16.5 ‰ and the annual average
448 $\delta^{13}\text{C}$ value was -20.9 ‰ (S.D. = ± 3.2 ‰). The annual average carbon to nitrogen (C:N) ratio of POM
449 was 7.0 ± 0.4 (average \pm S.D.), ranging from 6.8 to 7.7 (Table 34).

450 3.3. Seasonal variation of biochemical composition

451 The contents of CHO, PRT, and LIP of POM in the water column ~~ranged from~~ were $14.2 \mu\text{g}$
452 ~~L^{-1} to $412.3 \mu\text{g L}^{-1}$ ($129.5 \pm 87.2 \mu\text{g L}^{-1}$), from $22.8 \mu\text{g L}^{-1}$ to $382.4 \mu\text{g L}^{-1}$ ($155.0 \pm 73.3 \mu\text{g L}^{-1}$), and~~
453 ~~from $21.4 \mu\text{g L}^{-1}$ to $401.4 \mu\text{g L}^{-1}$ ($154.9 \pm 78.9 \mu\text{g L}^{-1}$), respectively (Table 4). The FM contents of~~
454 POM ranged from $170.9 \mu\text{g L}^{-1}$ to $915.7 \mu\text{g L}^{-1}$ ($435.5 \pm 175.5 \mu\text{g L}^{-1}$). ~~Since there were no significant~~
455 ~~differences in biochemical concentrations of POM and FM among 3 light depths and spatial~~
456 ~~distributions, On monthly basis, we averaged each biochemical compound and FM from every depths~~
457 ~~and stations (Fig. 2) on monthly basis. The biochemical compositions varied seasonally.~~ The CHO
458 and LIP concentrations increased from April to August and decreased from August to October in 2012.
459 In contrast, the PRT concentrations decreased from April to October in 2012 and increased from
460 October in 2012 to April in 2013. The seasonal pattern of FM concentrations was similar to the
461 pattern of chl *a* concentrations ($r = -0.36$, $p < 0.05$, Pearson's Correlation Coefficient).

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462 In order to estimate the biochemical composition as food quality, we obtained relative
463 contributions of each biochemical concentration of POM to FM, based on percentage basis. The
464 biochemical composition of each class (CHO, PRT and LIP) ~~ranged from~~ were 8.3% to 59.1% , ~~from~~

465 | 6.8% to 74.9% and from 9.4% to 68.3%, respectively (annual [meanaverage](#) \pm S.D. of CHO, PRT,
466 | and LIP composition = $26.4 \pm 9.4\%$, $37.8 \pm 16.1\%$, and $35.7 \pm 13.9\%$, respectively; Table 45). ~~We
467 | found the seasonal variation of biochemical composition based on monthly basis of biochemical
468 | composition (Fig. 2). To illustrate these variations of biochemical composition of POM, the highest
469 | and lowest PRT compositions were in April 2012 and August 2012. In contrast to PRT compositions,
470 | the highest and lowest LIP compositions were in August 2012 and April 2012. CHO composition was
471 | recorded high in January 2013, but to compare CHO composition to PRT and LIP composition, CHO
472 | composition was not strong varied during the study period.~~

473 | 3.4. Seasonal variations of the calorific values and contents of FM

474 | The calorific values and contents of FM ~~ranged from were~~ 5.4 Kcal g^{-1} to 7.9 Kcal g^{-1}
475 | (annual [meanaverage](#) \pm S.D. = $6.6 \text{ Kcal g}^{-1} \pm 0.6 \text{ Kcal g}^{-1}$) and 1.0 Kcal m^{-3} to 6.1 Kcal m^{-3} (annual
476 | [meanaverage](#) \pm S.D. = $2.8 \text{ Kcal m}^{-3} \pm 1.1 \text{ Kcal m}^{-3}$), respectively (Table 45). The calorific values of
477 | ~~FM~~ had no apparent seasonal pattern, whereas the calorific contents ~~of FM~~ had a seasonal pattern
478 | similar to the seasonal variation of FM concentrations.

479 | 3.5. Relationship between biochemical pools and environmental conditions

480 | Relationships between biochemical pools and environmental conditions were performed
481 | using Pearson's correlation matrix ([Table 6](#)). Based on the results, we found a significant, positive
482 | relationships between PRT composition and river-input ($r = 0.84$, $p < 0.01$, [Table 5](#), Fig. 3) and PRT
483 | composition and dissolved nitrogen concentrations (NH_4 : $r = 0.69$, $p < 0.01$; $\text{NO}_2 + \text{NO}_3$: $r = 0.54$, $p <$
484 | 0.01 , [Table 5](#)). Lipid composition had an inverse relationships with river-input ($r = -0.63$, $p < 0.01$)
485 | and dissolved nitrogen concentrations (NH_4 : $r = -0.59$, $p < 0.01$; $\text{NO}_2 + \text{NO}_3$: $r = -0.53$, $p < 0.01$).
486 | These relationships led to a significant reverse relationship between PRT composition and LIP
487 | composition ($r = -0.81$, $p < 0.01$, Fig. 4). PRT composition was negatively correlated with temperature
488 | ($r = -0.52$, $p < 0.01$), whereas LIP composition was positively correlated with temperature ($r = 0.72$, p

489 < 0.01). ~~There were no significant relationships between PRT composition and irradiance and LIP~~
490 ~~composition and irradiance.~~

491 **4. Discussion and conclusion**

492 **4. 1. Environmental conditions and chl-*a* concentration**

493 The annual average chl-*a* concentration during the research period ~~was 3.4 $\mu\text{g L}^{-1}$ (S.D.=~~
494 ~~$\pm 2.8 \mu\text{g L}^{-1}$) with a range from 0.8 to 14.2 $\mu\text{g L}^{-1}$ which~~ is in a similar range of chl-*a* concentrations
495 reported previously in Gwangyang Bay, although it varied across different seasons and sampling
496 depths (Cho et al., 1994; ~~Choi and Noh et al.~~, 1998; Lee et al., 2001a; Kwon et al., ~~2001~~2002; Jang
497 et al., 2005; Yang et al., 2005; Beak et al., 2011; Min et al., 2011; Beak et al., 2015). Previous studies
498 reported that chl-*a* concentration was influenced mainly by salinity, temperature, and nutrients (nitrate
499 and phosphate) depending on freshwater input from the Seomjin River. Our results in this study were
500 similar to former studies ($r = 0.34$ and -0.41 , $p < 0.05$, $n = 48$ and 28 for salinity and NH_4 ,
501 respectively). However, high chl-*a* concentrations were previously recorded in spring and fall,
502 whereas the highest concentrations were observed in summer (August 2012) from this study. In fact,
503 Baek et al. (2015) reported that high chl-*a* concentrations were found in summer similarly, although
504 there was difference between environmental factors and chl-*a* concentrations as compared with our
505 results. The high levels of chl-*a* were observed with high nutrient concentrations and low salinity
506 levels in the surface water by Baek et al. (2015), whereas the high values existed with low nutrient
507 concentrations and high salinity levels in our results.

508 Despite this dissimilarity of environmental factors with high chl-*a* concentrations, we also
509 found the highest chl-*a* concentrations observed in summer. According to Shaha and Cho (2009),
510 there is a tendency with increasing precipitation and river-input in Gwangyang Bay during summer.
511 This trend could increase loading nutrients from freshwater for maintaining phytoplankton growth in
512 summer. In addition, a strong light intensity during summer could be favorable for phytoplankton

513 growth since our study area was extremely turbid conditions during almost all seasons due to
514 freshwater discharge and a strong spring-neap tidal oscillation. As a result, the combination of these
515 factors is believed to enhance chl-*a* concentration and primary production of phytoplankton during
516 summer in Gwangyang Bay.

517 4. 2. POM characterization

518 In general, POM consists of a mixture of living as well as detritus materials (phytoplankton,
519 bacteria, zooplankton, fecal pellets, terrestrial matters, etc.) originating from freshwater and estuarine
520 and marine environments. POM samples can be characterized or determined for source of the major
521 contributor(s). The C:N ratio generally ranges between 6 and 10 for phytoplankton, whereas the ratios
522 are between 3 and 6 for zooplankton and bacteria (Savoie et al, 2003; references therein). For
523 terrestrial organic matters, the C:N ratios are normally over 12 (Savoie et al, 2003; references therein).
524 Therefore, it is useful to classify phytoplankton from heterotrophs and terrestrial materials (Lobbés et
525 al., 2000; Savoie et al., 2003; Lee and Whitley, 2005). In this study, the meanaverage C:N ratios of
526 POM was 7.0 (S.D. = ± 0.4), which indicates that this POM is mainly phytoplankton (Table 34).
527 However, the original C:N ratio ~~must can be used with caution~~ changed because of its variation in
528 caused by the process of POM degradation ~~biochemical alterations (Savoie et al, 2003)~~. For example,
529 PON is preferentially degraded compared to POC of phytoplankton, which causes an increase of the
530 C:N ratio (Thornton and McManus, 1994; Savoie et al, 2003). ~~In contrast, T~~terrestrial organic matters
531 ~~(with high C:N ratios)~~ colonized by bacteria ~~(with low C:N ratios)~~ could ~~lowers~~ their initial high C:N
532 ratio (Savoie et al, 2003; references therein). Therefore, similar C:N ratios of POM could be
533 produced by degraded phytoplankton and bacteria-colonized terrestrial organic matters (Lancelot and
534 Billen 1985; Savoie et al, 2003). In addition to C:N ratios, $\delta^{13}\text{C}$ of POM can be alternatively used for
535 determining their origin. Kang et al. (2003) reported that the meanaverage $\delta^{13}\text{C}$ signature of
536 phytoplankton in Gwangyang Bay was -20.8 ‰ (S.D. = ± 1.1‰). In this study, our meanaverage $\delta^{13}\text{C}$
537 signature of POM was -20.9 ‰ (S.D. = ± 3.2‰), which also indicates that POM was mostly

538 phytoplankton during the study periods (Table 34). However, some large contributions of benthic
539 microalgae were seasonally found in our samples with relatively higher $\delta^{13}\text{C}$ values on August and
540 October 2012 (Table 34). According to Kang et al. (2003), the ~~mean~~average $\delta^{13}\text{C}$ value of benthic
541 microalgae is approximately -14.1 ‰ in Gwangyang Bay. Based on our C:N ratio and $\delta^{13}\text{C}$ value in
542 this study, we confirmed that our POM samples were primarily comprised of phytoplankton
543 (seasonally benthic microalgae) in Gwangyang Bay. This is interesting that river-derived terrestrial
544 organic matters were not important component of the POM in Gwangyang Bay with a large river
545 runoff. Indeed, several previous studies reported a small fraction of terrestrial particulate matter in the
546 same bay as well as in the southeastern coastal bays in Korea (Kang et al., 1993; Lee et al., 2001b;
547 Kwon et al., 2002). Currently, we do not have solid mechanisms for the low contribution of terrestrial
548 organic matters. A further investigation is needed for this paradoxical process.

549 **4. 3. Environmental conditions and biochemical pools**

550 Biochemical pools of POM originating from phytoplankton are influenced by various
551 environmental factors, such as temperature, salinity, nutrients, and light conditions (Morris et al., 1974;
552 Smith and Morris, 1980; Rivkin and Voytek, 1987; Boëchat and Giani, 2008; Cuhel and Lean, 1987;
553 Mock and Kroon, 2002; Khotimchenko and Yakoleva, 2005; Ventura et al., 2008; Sterner et al. 1997).
554 In this study, significant relationships were found between environmental conditions and biochemical
555 pools, especially PRT and LIP (Table 5). Temperature was positively and negatively correlated with
556 LIP and PRT. Previous studies reported that higher temperature stress mainly affects nitrogen
557 metabolism (Kakinuma et al., 2006) which is related to significant decrease of PRT with increases of
558 LIP and CHO content (Tomaselli et al., 1988; Oliveira et al., 1999). In a high temperature-stressed
559 condition of phytoplankton, the decrease in PRT content is related to breakdown of protein structure
560 and interference with enzyme regulators (Pirt, 1975), whereas LIP is predominant because LIP is more
561 closely associated with cell structure such as thickened cell walls (Smith et al., 1989; Kakinuma et al.,
562 2001, 2006). Our results are in agreement with other works, as described above.

563 The relationships between nutrients and biochemical pools could be explained by nutrient
564 limitation and the characteristics of each biochemical compound. A combination of nutrient
565 concentrations and ratios can be used to assess nutrient limitation (Dortch and Whitledge, 1992; Justić
566 et al., 1995). Dortch and Whitledge (1992) suggested that nutrient limitations are existed in the
567 Mississippi river plume and Gulf of Mexico, if the dissolved inorganic phosphorus (DIP), dissolved
568 inorganic nitrogen (DIN), and dissolved silicon (DSi) concentrations in water column are less than 0.2,
569 1.0 and 2.0 μM , respectively, depending on the half-saturation constant (K_s) that the threshold value is
570 required for the uptake and growth of phytoplankton (Eppley et al., 1969; Fisher et al 1988). In
571 addition, molar ratios of the DIN:DIP ~~and~~, DSi:DIN and DSi:DIP can be indicators of nutritional
572 status and the physiological behavior of phytoplankton (Redfield et al., 1963; Goldman et al., 1979;
573 Elrifi and Turpin, 1985; Dorch and Whiteledge 1992; Roelke et al. 1999). According to Dortch and
574 Whitledge (1992), the following criteria of their molar ratios were (a) DIN:DIP ratio < 10 and
575 DSi:DIN ratio > 1 for nitrogen (N) limitation; (b) DIN:DIP ratio > 30 and DSi:DIP ratio > 3 for
576 phosphorus (P) limitation; (c) DSi:DIN ratio < 1 and DSi:DIP ratio < 3 for silicate (Si) limitation. In
577 this study, nutrient limitation conditions were observed by absolute nutrient concentrations or/and
578 their molar ratios depending on seasons (Table 62). Previous studies of biochemical composition in
579 relation to nutrient limitation reported that PRT production of phytoplankton was enhanced under
580 abundant N conditions (Fabiano et al., 1993; Lee et al., 2009). In contrast, LIP production and storage
581 were dominant (Shifrin and Chisholm, 1981; Harrison et al., 1990) and PRT contents decreased
582 (Kilham et al., 1997; Lynn et al., 2000; Heraud et al., 2005) under N-depleted conditions. High LIP
583 contents have also been detected in phytoplankton under P or/and Si limitation (Lombardi and
584 Wangersky, 1991; Lynn et al. 2000; Heraud et al., 2005; Sigee et al., 2007). Under N or P-limited
585 conditions, triglyceride content (energy storage) increases and shifts from PRT to LIP metabolism
586 since proteins are nitrogenous compounds whereas LIP and CHO are non-nitrogenous substrates
587 (Lombardi and Wangersky, 1991; Smith et al., 1997; Takagi et al., 2000). In our study, Si and P
588 concentrations may not significantly impact on biochemical composition of phytoplankton. Si

589 concentrations were almost above 2.0 μM except in April 2013 during the study period. P limitation
590 was observed based on the absolute concentration and molar ratios during study period. However,
591 under P limitation, phytoplankton can relocate the cellular P pool to maintain their P requirements for
592 the maximum growth rate (Cembella et al., 1984; Ji and Sherrell, 2008). In this respect, we suggest
593 that DIN could be significantly impact on biochemical composition of phytoplankton in our study
594 area. DIN was initially believed to be the most important limiting factor for phytoplankton growth in
595 marine ecosystems (Ryther and Dunstan, 1971; Howarth, 1988). In fact, DIN was strongly positively
596 correlated with PRT composition, whereas it was negatively correlated with LIP composition. The
597 most of DIN loading came from freshwater input of the Seomjin River (Table 56, river-input vs NH_4
598 and NO_2+NO_3 ; $r = 0.91$ and 0.55 , $p < 0.01$, respectively) influences on PRT and LIP synthesis and
599 subsequently macromolecular composition of phytoplankton. As a result, the amount of river-input
600 was also strongly correlated with PRT composition (Table 56 and Fig. 3). Therefore, DIN is an
601 important controlling factor for biochemical composition, especially PRT and LIP composition of
602 phytoplankton in Gwangyang Bay.

603 Although irradiance is also known for an important governing factor for biochemical
604 composition, irradiance was not ~~significantly-statistically~~ correlated with biochemical pools in this
605 study (Table 56). We measured ~~irradiance-PAR~~ during our short incubation time (4~5h) for
606 phytoplankton productivity as a parallel study. Since this short time of measured irradiance can be
607 largely variable by a local weather, it might be not enough to reflect and detect the change of
608 biochemical composition in phytoplankton with irradiance. The irradiance between April 2012 and
609 April 2013 was largely different (approximately 10 times lower in April 2012 than in April 2013;
610 Table 1). Increasing synthesis of proteins is found as light intensity decrease because a relatively
611 lower irradiance saturation level is required for protein synthesis than that of other biochemical
612 components (Lee et al., 2009; Suárez and Marañón, 2003; Morris et al., 1974, 1978). Consistently, the
613 protein compositions were significantly higher in April 2012 than in April 2013 (t -test, $p < 0.01$; Fig.

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614 2) in this study. The proteins accounted approximately 62 % and 37 % of biochemical compositions in
615 April 2012 and April 2013, respectively. However, the main reason for no consistent relationships
616 between irradiance and biochemical components along seasons might be the PAR measurements as
617 discussed previously in this study.

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

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

634 **4.4. Total FM and energy content of POM in a global context**

635 ~~The annual average of FM was $434.5 \mu\text{g L}^{-1}$ (S.D. = $\pm 175.5 \mu\text{g L}^{-1}$) in this study.~~ Since there
636 were no comparable data available in South Korea, we compared our results with other regions (Table
637 7), although they were conducted in different seasons and sampling depths. PRT contents in this study

638 were as high as in the Ross Sea (Fabiano and Pusceddu, 1998; Fabiano et al., 1999a), the Amundsen
639 Sea (Kim et al., ~~2015~~2016) and the Humboldt Current System (Isla et al., 2010). A similar range of
640 LIP contents was observed in Bedford Basin (Mayzaud et al., 1989), Yaldad Bay (Navarro et al., 1993)
641 and the Humboldt Current System (Isla et al., 2010). CHO contents were comparatively higher in this
642 study than other studies except Bedford Basin (Mayzaud et al., 1989) and Yaldad Bay (Navarro et al.,
643 1993). One of the highlights is that the calorific contents of FM in this study were generally higher
644 than those of other areas except several regions. The FM values were comparatively higher than other
645 regions such as the northern Chuekchi Sea (Kim et al., ~~2014~~2015; Yun et al., ~~2014~~2015), Ross Sea
646 (Fabiano et al., 1996; Fabiano and Pusceddu, 1998; Fabiano et al., 1999a; Pusceddu et al., 1999),
647 Amundsen Sea (Kim et al., ~~2015~~2016) and the northern part of the East/Japan Sea (Kang et al.,
648 unpublished) or similar to the Humboldt Current System which is known as an important spawning
649 sites for pelagic fishes and the highest abundance of anchovy eggs (Isla et al., 2010). Actually, the
650 southern coastal sea (including our study area) in Korea represents calm seas, an indented coastline,
651 and numerous bays, which have high diversities of habitat for fishes and shellfishes (Kwak et al.,
652 2012) and give a favorable condition for mariculture (Kwon et al., 2004). The high quantity of FM
653 and the calorific contents of POM found in this study reflected good nutritive conditions of primary
654 food materials mainly provided by phytoplankton for the maintenance of productive shellfish and fish
655 populations in Gwangyang Bay.

656 ~~5. Summary and Conclusion~~

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

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

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

681

682 **Acknowledgements**

683 ~~We thank the anonymous reviewers who greatly improved an earlier version of the~~
684 ~~manuscript.~~ This research was supported by "Long-term change of structure and function in marine
685 ecosystems of Korea" funded by the Ministry of Oceans and Fisheries, Korea.

686

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- 서식 있음: 글꼴: (한글) 굴림, 프랑스어(프랑스)
- 서식 있음: 프랑스어(프랑스), 강조 없음
- 서식 있음: 글꼴: (한글) 굴림, 프랑스어(프랑스)
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- 서식 있음: 글꼴: (한글) 굴림, 프랑스어(프랑스)
- 서식 있음: 글꼴: (한글) 굴림

974 **Table captions**

975 Table 1. Environmental factors and chl-*a* concentrations in Gwangyang bay during the research period

976 (- : no data).

977 Table 2. Monthly patterns of Rrainfall and river input.

978 Table 3. $\delta^{13}\text{C}$ values and C:N ratios of POM at surface in Gwangyang bay ~~(surface)~~.

979 Table 4. Biochemical concentrations and composition, calorific values and contents in Gwangyang

980 bay (- : no data).

981 Table 5. Significant correlation coefficient (*r*) among proteins (PRT), lipids (LIP) and environmental

982 factors (ns ; no significance, **: $p < 0.01$). River-inputs were integrated from 20 days prior to

983 our sampling dates.

984 Table 6. Observed nutrient limitations during the study period.

985 Table 7. Comparison of biochemical quantity of POM, FM and the calorific contents.

986 **Figure captions**

987 Fig. 1. Sampling location in Gwangyang bay, Korea ; Maps of Korea (a), Southern Coastal Sea (b)
988 and main sampling stations (c).

989 Fig. 2. Seasonal variation of biochemical composition in Gwangyang bay.

990 Fig. 3. The positive relationship between river-input and protein composition. River-inputs were
991 integrated from 20 days prior to our sampling dates.

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