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1 Surface zooplankton size and taxonomic composition in Bowdoin Fjord, north-western  
2 Greenland: A comparison of ZooScan, OPC and microscopic analyses

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17 **Abstract**

18 In Greenland, tidewater glaciers discharge turbid subglacial freshwater into fjords,  
19 forming plumes near the calving fronts. To evaluate the effects of this discharge on the  
20 zooplankton community in the fjords, we collected sea surface zooplankton samples in  
21 Bowdoin Fjord in north-western Greenland during the summer of 2016 and made  
22 microscopic, OPC and ZooScan analyses. Within the three quantitative methods,  
23 ZooScan has advantages that can evaluate various parameters (e.g., abundance, biomass,  
24 size and taxonomic information) simultaneously and has the ability to eliminate abiotic  
25 particles, such as silt and sediment, which are abundant in samples. Based on taxonomic  
26 biomass data, the zooplankton community is clustered into three groups, which varied  
27 spatially: inner, middle and outer fjord groups. Jellyfish dominated the outer fjord group,  
28 and barnacle cypris larvae dominated the middle fjord group. For the inner fjord group,  
29 large-sized *Calanus* spp. and chaetognaths were abundant. Since these species are  
30 characterized with oceanic taxa, they would intrude through the deep fjord water and  
31 subsequently be upwelled through entrainment of glacially modified plume water. From  
32 the NBSS analysis on zooplankton size spectra, the steep slope of NBSS in the middle  
33 fjord community suggests that the high productivity was caused by the addition of  
34 meroplanktonic cypris larvae.

35

36 **Keywords**

37 glacial fjord, Bowdoin Fjord, zooplankton, ZooScan, NBSS

38

## 39 **1. Introduction**

40 Recently, tidewater glaciers in Greenland have been thinning and retreating under the  
41 influence of atmospheric warming (e.g., Howat and Eddy, 2011; Murray et al., 2015).  
42 These glaciers flow directly into the ocean, forming an important ice-ocean boundary in  
43 a glacial fjord. Near the glacier front, subglacial discharge upwells and forms  
44 a sediment-rich turbid meltwater plume (Chu, 2014; Ohashi et al., 2016; Kanna et  
45 al., 2018). In front of tidewater glaciers, particularly near the plume, dense occurrences  
46 of marine mammals and sea birds are commonly observed (Hop et al., 2002; Lydersen et  
47 al., 2014; Dalpadado et al., 2016; Arimitsu et al., 2016). These aggregations of marine  
48 mammals and sea birds at meltwater plumes in glacial fjords suggest that their food,  
49 especially zooplankton, may be higher than in other regions. However, sampling and  
50 measurements are difficult near the glacier front; thus, little information on zooplankton  
51 abundance is available in glacial fjords near the plumes.

52 For the evaluation of zooplankton, size and taxa are two important proxies to  
53 evaluate their quantitative and qualitative roles. Using size spectra zooplankton biomass  
54 data, calculation of normalized biomass size spectra (NBSS) provides valuable  
55 information on zooplankton productivity, energy transfer efficiency and their prey-  
56 predator linkages (Zhou, 2006; Zhou et al., 2009). While important, size measuring  
57 zooplankton by microscopic observations is time consuming. Taxonomic identification  
58 under a microscope also requires knowledge of zooplankton taxa. To overcome these  
59 problems, several instruments have been developed. An Optical Plankton Recorder  
60 (OPC) using light attenuation is an instrument that can quantify zooplankton in 4096 size  
61 categories between 0.25 and 5.0 mm using the Equivalent Spherical Diameter (ESD) in a  
62 short time (Herman, 1988, 1992). While useful, the OPC does not provide taxonomic

63 information. An instrument that measures both size and taxonomic information at the  
64 same time, the zooplankton scanning image analysis system (ZooScan) was established  
65 (Gorsky et al., 2010). ZooScan has been used in various locations (e.g., Abrolhos Bank,  
66 Bay of Biscay, off Ubatuba, Brazil and others) (Marcolin et al., 2013, 2015; Vandromme  
67 et al., 2014). However, little information is available for inter-calibration with other  
68 instruments, which prevents the evaluation of measurement characteristics of the  
69 ZooScan.

70 In the present study, we studied the size and taxonomic composition of sea-  
71 surface zooplankton in Bowdoin Fjord, a glacial fjord located in north-western Greenland  
72 during July 2016. Zooplankton samples, collected by sea-surface tow at fifteen stations  
73 set from the plume to the outside along with fjord, were preserved. Using the same  
74 zooplankton samples, their size spectra were quantified by OPC and ZooScan, and  
75 taxonomic accounts were also identified with ZooScan and microscopic observations.  
76 Applying size-mass relationships, zooplankton biomass (wet mass: WM) derived by OPC  
77 and ZooScan were compared with directly measured WM. Finally, the calculation of  
78 NBSS based on OPC and ZooScan may enable us to evaluate the measurement  
79 characteristics (causes of under/over estimation) of each instrument and the regional  
80 characteristics of zooplankton in the glacial fjord.

## 81 **2. Materials and methods**

### 82 *2.1. Field sampling*

83 Bowdoin Glacier (77°41'N, 68°35'W) is a marine-terminating glacier located along the  
84 coast of Prudhoe Land in north-western Greenland (Sakakibara and Sugiyama, 2018).  
85 The glacier flows into Bowdoin Fjord at a rate of ~500 m year<sup>-1</sup> and discharges icebergs

86 and meltwater through a 3-km wide calving front (Fig. 1a) (Sugiyama et al., 2015). The  
87 glacier is ~280 m thick and the fjord is ~250 m deep near the calving front. Boat-based  
88 observations were made in the daytime during 27-29 July 2016. Temperature and  
89 salinity at 2 m were measured with a CTD profiler (ASTD 102, JFE Advantech, Japan)  
90 at 44 stations, which encompassed the plume through to the outside of the fjord (data  
91 from Kanna et al., 2018). At 15 stations, a horizontal tow of a single-NORPAC net  
92 (mouth diameter 45 cm, mesh size 335  $\mu\text{m}$ ) at 2-3 m was made over three minutes. To  
93 register the filtered water volume, a flowmeter (Rigotsha, Saitama, Japan) was mounted  
94 in the mouth of the net. The net sampling depth was also monitored by a depth recorder  
95 (DEFI2-D50, JFE Advantech, Japan). Zooplankton samples were preserved with borax-  
96 buffered formalin by adding 5% volume to the total zooplankton samples.

## 97 *2.2 Microscopic observation and wet mass measurement*

98 In the laboratory, microscopic observations were made of subsamples (1/4 to 1/32) made  
99 by a Motoda splitter (Motoda, 1959) according to the size of samples. Species and  
100 taxonomic identifications, sorting and counting were made under a stereomicroscope  
101 (Nikon SMZ800N). Taxa except copepods were counted with taxon (e.g. jellyfishes,  
102 chaetognaths, appendicularians, euphausiids, polychaetes, barnacles). For copepods,  
103 classification of *Calanus* spp. and other species was made. The sorted samples were  
104 placed on pre-weighed mesh (100  $\mu\text{m}$ ), seawater was removed with aid of tissue, then the  
105 wet mass (WM) was measured with a microbalance (Mettler Toledo AE100) with the  
106 precision of 0.1 mg. All abundance and biomass data are shown as per cubic metre (ind.  
107  $\text{m}^{-3}$  or mg WM  $\text{m}^{-3}$ ).

108 *2.3 OPC measurement*

109 OPC measurements were made with a bench-top OPC (Model OPC-1L: Focal  
110 Technologies Corp.) using 1/2-1/128 subsamples (varied according to the size of the  
111 samples) of the total formalin-preserved samples. OPC measurements were made at a  
112 low flow rate (ca. 10 L min<sup>-1</sup>) and low particle density (<10 counts s<sup>-1</sup>) without staining  
113 (Yokoi et al., 2008).

114 The abundance per cubic metre ( $N$ : ind. m<sup>-3</sup>) for each of the 4,096 ESD size  
115 categories was calculated using the following equation:

116 
$$N = \frac{n}{s \times F}$$

117 where  $n$  is the number of particles (=zooplankton ind.),  $s$  is the split factor of each sample,  
118 and  $F$  is the filtered volume of the net (m<sup>3</sup>). The biovolume of the zooplankton  
119 community in 4,096 size categories was calculated from the ESD data, and the biovolume  
120 (mm<sup>3</sup> m<sup>-3</sup>) was calculated by multiplying  $N$  and the volume (mm<sup>3</sup> ind.<sup>-1</sup>) derived from  
121 ESD. Zooplankton wet mass (WM) for 4,096 size categories was calculated from the  
122 ESD data by assuming the relative density of zooplankton to be equal to that of seawater  
123 (1 mg mm<sup>-3</sup>). Analyses of the mesozooplankton biomass were performed with the  
124 separation of five size classes (0.335-1 mm, 1-2 mm, 2-3 mm, 3-4 mm, 4-5 mm ESD).

125 *2.4 ZooScan measurement*

126 Zooplankton images were scanned with a water-proof ZooScan (ZooScan MIII,  
127 Hydroptic Inc., France) using 1/4-1/128 subsamples (varied according to the size of the  
128 samples) of the total formalin-preserved samples. The overall process and analysis  
129 followed Gorsky et al. (2010). Before each measurement, background measurements  
130 were made by filling with deionized water. ZooScan measurements were made under

131 the condition that all zooplankton sank to the bottom of a scanning cell of 15 cm × 24 cm  
132 in area. Zooplankton overlapping was avoided by using soft tweezers manually.

133 The obtained zooplankton images were separated from individual objects by the  
134 ZooProcess software. Zooplankton images were digitalized at 2400 dpi resolution.  
135 From this resolution, one pixel corresponded to 10.58 μm. For identification, all  
136 obtained images were uploaded to the website EcoTaxa (<http://ecotaxa.obs-vlfr.fr/prj/>).  
137 Images, identified as “detritus”, “fibre”, “artefact” and “other”, were removed for further  
138 analyses. ZooScan provides estimates of body length (major axis of the best fitting  
139 ellipse) and width (minor axis) (Gorsky et al., 2010). From these major and minor axes,  
140 the biovolume was calculated:  $\text{biovolume} = 4/3 \times \pi \times (\text{major axis})/2 \times (\text{minor axis}/2)^2$ .  
141 From these biovolume data, the equivalent spherical diameter (ESD, μm) was computed  
142 for each zooplankton object. Zooplankton wet mass (WM) was calculated from the ESD  
143 data by assuming the relative density of zooplankton to be equal to that of seawater (1 mg  
144 mm<sup>-3</sup>).

## 145 *2.5 Data analysis*

146 To evaluate regional changes in the zooplankton community, a cluster analysis based on  
147 biomass was performed. Zooplankton biomass data (*ZB*: mg WM m<sup>-3</sup>) of each taxon  
148 (jellyfishes, chaetognaths, appendicularians, euphausiids, polychaetes, barnacles,  
149 *Calanus* spp. and other copepods) were normalized as  $\log_{10}(ZB+1)$ . Next, similarities  
150 between zooplankton samples were calculated using the Bray-Curtis similarity index.  
151 To group the samples, similarity indices were coupled with hierarchical agglomerative  
152 clustering using a complete linkage method (Unweighted Pair Group Method using  
153 Arithmetic mean: UPGMA; Field et al., 1982). These analyses were made with



154 PRIMER v7 (PRIMER-E Ltd.).

155 From OPC and ZooScan data, zooplankton biovolume ( $\text{mm}^3 \text{m}^{-3}$ ) between 0.335-  
156 5.0 mm ESD was summed at each 0.1-mm ESD size class interval. To calculate the  $X$ -  
157 axis of the NBSS ( $X$ :  $\log_{10}$  zooplankton biovolume [ $\text{mm}^3 \text{ind.}^{-1}$ ]), the biovolume was  
158 divided by the abundance of each size class ( $\text{ind. m}^{-3}$ ) and converted to a common  
159 logarithm. To calculate the  $Y$ -axis of the NBSS ( $Y$ :  $\log_{10}$  zooplankton biovolume [ $\text{mm}^3$   
160  $\text{m}^{-3}$ ]/ $\Delta$ biovolume [ $\text{mm}^3$ ]), the biovolume was divided by the interval of biovolume  
161 ( $\Delta$ biovolume [ $\text{mm}^3$ ]) and converted to a common logarithm. Based on these data, the  
162 NBSS linear model was calculated as follows:

$$163 \quad Y = aX + b$$

164 where  $a$  and  $b$  are the slope and intercept of the NBSS, respectively.

165 To make comparisons of NBSS between OPC and ZooScan, a  $U$ -test was made.  
166 To evaluate whether the NBSS slope varied with the NBSS intercept or measured  
167 instruments, an analysis of covariance (ANCOVA) with the NBSS intercept and measured  
168 instruments (OPC or ZooScan) as independent variables was conducted.  $U$ -test analyses  
169 and an ANCOVA were performed using StatView v5 (SAS Institute Inc., Cary, NC, USA).

### 170 **3. Results**

#### 171 *3.1. Hydrography*

172 Temperature at the 2-m depth ranged between 3.16-5.21°C, and decreased from the  
173 glacier to outer fjord, while it showed abrupt high temperatures at the farthest offshore  
174 station (Fig. 1b). Salinity was in the range of 13.62 to 30.52 and increased from the  
175 glacier plume to the outer fjord.

176 *3.2. Calibration*

177 Comparisons of abundance (ind. m<sup>-3</sup>) and WM (mg WM m<sup>-3</sup>) between OPC or ZooScan-  
178 derived data and direct measurements were made (Fig. 2). Based on whole samples ( $n$   
179 =15), all measurements were highly correlated with each other ( $r^2=0.86-0.95$ ,  $p<0.0001$ ).  
180 For abundance, both OPC and ZooScan underestimated more than microscopic  
181 observations (by a factor of 0.71-0.78), while OPC:ZooScan had similar values (0.91)  
182 (Fig. 2a). For biomass, while coefficients of determination were high ( $r^2=0.90-0.95$ ),  
183 differences due to measurement methods were greater than those for abundance. Thus,  
184 within the same samples, ZooScan quantified the largest value, followed by direct  
185 measurements, and OPC yielded the least value (Fig. 2b).

186 *3.3. Cluster analysis*

187 Based on directly measured zooplankton biomass, the zooplankton community was  
188 separated into three groups (A, B and C) at 47.5% similarity (Fig. 3a). The horizontal  
189 distribution of each group varied clearly (Fig. 3b). Thus, group A was mainly observed  
190 in the outer fjord, while group B was concentrated at the centre of the plume in front of  
191 the glacier. The other largest group C mainly occupied the middle of the fjord. For  
192 each group, the dominant zooplankton taxa varied: jellyfishes dominated in group A,  
193 chaetognaths and copepods dominated in group B, and barnacle larvae (cypris) dominated  
194 in group C (Fig. 4).

195 *3.4. Inter-method comparison in taxa (ZooScan vs microscope)*

196 Within the three quantitative methods (OPC, ZooScan and microscope), taxonomic  
197 information was obtained from the ZooScan and by the microscope. Subsequently,

198 combining directly measured WM with taxa, the taxonomic composition of zooplankton  
199 abundance and biomass were compared between those from ZooScan and direct  
200 quantification. The abundance showed little differences between direct measurement  
201 and ZooScan (Fig. 4). On the other hand, biomass showed  
202 overestimation/underestimation, which varied with taxa and station (Fig. 4).

203 Abundance showed significant correlations between ZooScan and microscopic  
204 observations for all species/taxa, while biomass showed significant correlations for only  
205 four taxa, which accounted for half of the eight taxa (Fig. 5). In detail, for abundance,  
206 appendicularians, euphausiids and copepods (*Calanus* spp. and others) had nearly linear  
207 ( $Y:X=0.97-1.03$ ) correlations between the ZooScan and microscopic observations, while  
208 jellyfishes, chaetognaths, polychaetes and barnacle larvae were underestimated by  
209 ZooScan, with factors of 0.29-0.78 (Fig. 5). For biomass, while four taxa showed  
210 significant correlations between ZooScan and direct measurements, their factors in  
211 ZooScan were overestimations (1.26-2.94) for polychaete and barnacle larvae and  
212 underestimations (0.22-0.33) for appendicularians and other copepods.

### 213 3.5. Inter-method comparison in size (ZooScan vs. OPC)

214 Zooplankton size properties in abundance and biomass were quantified by two methods:  
215 OPC and ZooScan. For abundance, both methods showed the predominance of the  
216 smallest size class (0.335-1 mm ESD) throughout the stations and had little differences  
217 with quantitative methods (Fig. 6). On the other hand, for biomass, differences between  
218 methods were detected. Thus, zooplankton group A was dominated by the small size  
219 class in OPC, while it was dominated by the large size class in ZooScan. For  
220 zooplankton group C, the opposite pattern was seen: i.e., dominance of the large size class

221 in OPC, while predominance of the small size class in ZooScan was observed (Fig. 6).

222 Comparison within size classes showed that the smallest size class (0.335-1 mm  
223 ESD) was highly correlated between ZooScan and OPC both in abundance and biomass  
224 (Fig. 7). For the other size classes, significant correlations were observed for the 2-3  
225 mm size class in abundance and the 1-2 and 2-3 mm size classes in biomass. Common  
226 patterns for these size classes were underestimations of ZooScan compared to OPC, with  
227 factors of 0.21-0.28 (Fig. 7).

### 228 3.6. Inter-method comparison in NBSS (ZooScan vs OPC)

229 The results of the NBSS analysis based on OPC and ZooScan are shown in Table 1.  
230 Slopes of NBSS based on OPC were -1.705 to -0.737 (mean±sd: -1.111±0.301) and those  
231 by ZooScan were -1.516 to -0.229 (-0.778±0.394). Slopes of NBSS were more  
232 moderate for ZooScan than those from OPC (*U*-test, *p*<0.05) (Fig. 8). Intercepts of  
233 NBSS based on OPC were -1.306 to -0.245 (-0.736±0.352), and those by ZooScan were  
234 -1.326 to -0.889 (-0.726±0.537). No significant differences were detected for intercepts  
235 of NBSS between OPC and ZooScan (*U*-test, *p*=0.958). From NBSS plots, it was  
236 notable that zooplankton biovolumes at smaller size classes were lower for ZooScan than  
237 for OPC (Fig. 8). This was due to the elimination of abiotic particles (e.g., silt or sand)  
238 from ZooScan data based on the imaging analysis. For NBSS slopes, an ANCOVA  
239 analysis, applying NBSS intercepts and differences in instruments (OPC or ZooScan) as  
240 the independent variables, detected significant differences only for the differences in the  
241 instruments (Table 2).

## 242 4. Discussion

243 Through zooplankton sample analyses based on multiple methods (i.e., microscope, WM,  
244 OPC and ZooScan measurements), various characteristics in the zooplankton community  
245 in the glacial fjord were evaluated. In the following section, we discuss three  
246 methodological notes in terms of taxonomic (ZooScan vs microscope), size and NBSS  
247 (ZooScan vs OPC) first. Then, we discuss the factors governing zooplankton  
248 community in the glacial fjord.

#### 249 *4.1. Taxonomic comparison*

250 While important, taxonomic information of zooplankton was not quantified in optical  
251 instruments such as OPC and LOPC (Herman, 1988; Nogueira et al., 2004). ZooScan  
252 obtains images of zooplankton and quantifies both size and taxonomic data from images  
253 (e.g., Vandromme et al., 2012). In this section, we compare taxonomic data from  
254 ZooScan with microscopic counts (abundance) and direct WM measurements (biomass).

255 Zooplankton abundance based on ZooScan have pointed out the possibility of  
256 underestimation of minor taxa/species due to the deviation or split of the samples (Colas  
257 et al., 2018). As a quantification method for minor and large-sized species/taxa, gently  
258 sieving through a 0.5-mm mesh and ZooScan measurements for each fraction have been  
259 proposed (Grosjean et al., 2004). However, it requires twice the time and is difficult to  
260 achieve for many samples (Colas et al., 2018). Underestimation of abundance due to  
261 the use of subsamples is reported to be common for rare species/taxa (Gorsky et al., 2010).  
262 This is considered to be the cause of the underestimation of abundance for two less  
263 abundant taxa: jellyfishes and chaetognaths in this study (Fig. 5), while underestimations  
264 for numerical dominant two taxa, polychaetes and barnacle larvae, would be caused by  
265 the double-splitting effects (note that the splitting was available for both microscopic

266 counts and ZooScan measurements) or heterogeneous distribution of these taxa in the  
267 samples.

268           For zooplankton abundance, a good correlation has been reported for automated  
269 and manual analyses based on same images created by ZooScan (Gorsky et al., 2010),  
270 while for zooplankton biomass, comparison between estimated values from ZooScan  
271 measurements and directly measured mass have not been reported to date. For biomass  
272 estimation using ZooScan, underestimation tends to occur for large-body sized organisms  
273 and taxa through the use of subsamples, since they are rare (e.g., termed “subsample  
274 effect”, cf. Colas et al., 2018). Fluctuations of ZooScan-derived biomass of  
275 chaetognaths and euphausiids in this study would be caused by this subsample effect (Fig.  
276 5). Interestingly, clear underestimation by ZooScan for appendicularian biomass  
277 occurred in this study (Fig. 5). This may due to the shape of appendicularian tails, which  
278 are often curved, affecting the automated measurements (Gorsky et al., 2010). The  
279 transparency of appendicularians may also effect the underestimation of their biomass  
280 (Herman, 1992). Concerning jellyfishes, the destruction of fragile organisms and  
281 species-specific differences in colour (=differences in transparency) may affect size  
282 measurements (Thompson et al., 2013). While a significant correlation pattern was not  
283 detected in this study, zero or overestimation were the cases for jellyfishes (Fig. 5). This  
284 may due to the underestimation by transparent body or overestimation of size due to the  
285 extension of jellyfish bodies on the measurement frame of ZooScan. For polychaetes  
286 and barnacles, the two overestimated taxa for ZooScan biomass, since most of them were  
287 small sized meroplanktonic larvae, it is difficult to accurately measure the wet mass using  
288 this method in this study. Thus, underestimation of directly measured wet mass would  
289 be the case for these two taxa.

290 4.2. *Size comparison*

291 In this study, the size property of zooplankton was measured by OPC and ZooScan.  
292 OPC measures the size of plankton by detecting the shade of planktonic particles created  
293 by a light beam and quantified size with 4,096 size units (Herman, 1992). Since OPC  
294 detects particle as shadows during flow through a channel, there are some potential  
295 sources of underestimation and overestimation on counting or sizing. As the cause of  
296 underestimation, underestimation in number by particle coincidence and underestimation  
297 in size caused by zooplankton direction to the light beam or body transparency are argued  
298 (Herman, 1992; Sprules et al., 1998; Zhang et al., 2000). Conversely, as the cause of  
299 overestimation, overestimation in number by counting on non-zooplankton particles, such  
300 as detritus and fragmentation of zooplankton body and overestimation in size by particle  
301 coincidence, are reported (Sprules et al., 1998; Zhang et al., 2000). For ZooScan, since  
302 ZooScan quantifies the biovolume of zooplankton by assuming a perfect spheroid shape,  
303 their estimated biomass is reported to be constantly higher than those measured by OPC  
304 (Schultes and Lopes, 2009). With such shortcomings, zooplankton biomass estimation  
305 by ZooScan is preferred to those of OPC because of the elimination of the aforementioned  
306 various under- and overestimations, which are inevitable for OPC measurements  
307 (Schultes and Lopes, 2009).

308 In the present study, correlations between OPC and ZooScan were detected for  
309 both abundance and biomass of the relatively small size classes (0.335-3 mm) (Fig. 7).  
310 Within them, highly significant correlations ( $r^2=0.99$ ,  $p<0.0001$ ) were observed for the  
311 smallest size fraction (0.335-1 mm). In detail, the abundance showed nearly an equal  
312 factor between them (1.04), while the biomass of ZooScan was 1.44 times higher than

313 that of OPC. This discrepancy would be caused by the differences in the biovolume  
314 measurement method in ZooScan (by assuming a perfect spheroid shape) mentioned  
315 above (Schultes and Lopes, 2009), while in the 1-3 mm size classes, a considerable  
316 underestimation by ZooScan (with a factor of 0.21-0.28 of OPC) occurred for both  
317 abundance and biomass (Fig. 7). For OPC, overestimations of abundance and biomass  
318 frequently occurred by including detritus count (overestimation in abundance) and size  
319 measurements on overlapping particles through a light beam (overestimation in biomass)  
320 (Sprules et al., 1998; Zhang et al., 2000). On the other hand, ZooScan can avoid the  
321 overlapping of particles by manual manipulation before measurement and can remove  
322 detrital material data through image analysis. In fact, we confirmed that detrital  
323 materials were composed  $88.2\pm 12.7\%$  of total particles (including both plankton and  
324 detritus) in 1-3 mm size classes by ZooScan image analysis. Thus, removing data on  
325 detrital materials may cause a greater underestimation of ZooScan than the data of OPC,  
326 which includes whole particles (both plankton and detritus). These facts suggest that  
327 ZooScan can provide more accurate data on plankton. Because of the lack of correlation  
328 detected for large size classes (3-5 mm), since these large size classes contain few  
329 individuals, variations due to sample split may induce the great variability in the  
330 individuals who belong to these size classes.

#### 331 4.3. NBSS

332 NBSS is a calculated linear expression based on zooplankton size and is treated as an  
333 index of the status of marine ecosystems (Herman and Harvey, 2006; Marcolin et al.,  
334 2015). The slope of NBSS represents zooplankton productivity, energy transfer  
335 efficiency and their prey-predator linkages (Zhou, 2006; Zhou et al., 2009). The



336 intercept of NBSS is an index of standing stocks (Sprules and Munawar, 1986). The  
337 slope of NBSS at approximately -1 indicates a theoretical steady state (Sprules and  
338 Munawar, 1986). In general, slopes steeper than -1 indicate bottom-up control (Moore  
339 and Suthers, 2006), or high productivity with low transfer efficiency (Sprules and  
340 Munawar, 1986; Zhou, 2006). Slopes flatter than -1 indicate top-down control (Moore  
341 and Suthers, 2006), or low productivity with high transfer efficiency (Sprules and  
342 Munawar, 1986; Zhou, 2006).

343           Since the ZooScan analysis is based on images, abiotic materials such as silt and  
344 sand are able to be eliminated before analysis (Gorsky et al., 2010). On the other hand,  
345 OPC could not separate plankton and abiotic particles. From the NBSS slope  
346 comparison between ZooScan and *in situ* LOPC in the Bay of Biscay, Vandromme et al.  
347 (2014) reported that the NBSS slope by LOPC (mean $\pm$ 1 sd:  $-0.97\pm 0.24$ ) is steeper than  
348 those by ZooScan ( $-0.86\pm 0.40$ ), while in a similar comparison in the Abrolhos Bank,  
349 Marcolin et al. (2013) reported the opposite pattern: i.e., the NBSS slope of LOPC is  
350 slightly flatter than those of ZooScan. These differences would be caused by the  
351 differences in the dominant zooplankton taxa, community structure and treated size  
352 ranges. In the present study, we applied the same size ranges for the NBSS calculation  
353 of both ZooScan and OPC and eliminated abiotic particle data for the NBSS calculation  
354 of ZooScan. This approach provides high biomass values for OPC, especially at small  
355 sizes, and steeper slopes for OPC ( $-1.11\pm 0.30$ ) than those of ZooScan ( $-0.78\pm 0.39$ ) (Fig.  
356 8).

357           For the intercept of NBSS, because of the overestimation in fragmentation of  
358 jellyfishes during course of quantification, *in situ* LOPC tends to overestimate more than  
359 those of ZooScan (Vandromme et al., 2014). In this study, the intercept of NBSS showed

360 no significant differences between those from OPC (mean±1 sd: -0.74±0.35) and  
361 ZooScan (-0.73±0.54) (*U*-test, *p* = 0.958). This finding may be observed because the  
362 abiotic particles eliminated from ZooScan analysis were mostly at smaller sizes, and their  
363 elimination had little effect on the NBSS intercept. Commonly, the NBSS intercept is  
364 correlated with the NBSS slope (cf. Matsuno et al., 2012). However, in this study, the  
365 NBSS slope has no correlation with the intercept, but has a correlation with the instrument  
366 (e.g., OPC or ZooScan) (Table 2). These facts suggest that the effect of elimination of  
367 abiotic particles in the ZooScan analysis may have a greater effect on the results of the  
368 NBSS slope. Thus, to make an accurate evaluation of the NBSS slope, NBSS  
369 calculations based on ZooScan, including elimination of abiotic particles, are  
370 recommended.

#### 371 4.4. Zooplankton community in the fjord

372 For zooplankton community, it should be noted that our data is only a surface sampling.  
373 Data interpretation by the differences of the sampling method from the previous studies  
374 should be considered. In glacial fjords, the zooplankton community is known to be  
375 strongly affected by the advection of outer oceanic water (Aksnes et al., 1989). For  
376 instance, the composition of the oceanic copepod *Calanus* spp. is increased through the  
377 outer fjord and accounted for 90% of their biomass (Arendt et al., 2010). In the present  
378 study, zooplankton biomass in the outer fjord (group A) was dominated by jellyfishes,  
379 and the composition of *Calanus* spp. was low (0.56-9.59%) (Figs. 3, 4). For this reason,  
380 the sampling methods of this study (horizontal net tow at sea surface [2-3 m]) may also  
381 be considered. Concerning jellyfishes, the dominance of the jellyfishes for the water  
382 masses outside the fjord are reported (Palma et al., 2014). Bearing this in mind, the

383 dominance of jellyfishes outside the fjord yield their dominance for zooplankton  
384 community group A.

385 For the zooplankton community in the middle of the fjord in Greenland, various  
386 copepods were dominant: *Metridia longa*, *Pseudocalanus* spp., *Microsetella* spp. and  
387 *Oncaea* spp. have been reported (Arendt et al., 2010; Swalethorp et al., 2015). However,  
388 in the present study, the zooplankton biomass of group C, observed in the middle of the  
389 fjord, was dominated by barnacle cypris larvae (Figs. 3, 4). Concerning cypris larvae in  
390 the fjord environment, Swalethorp et al. (2015) reported that cypris larvae were  
391 abundant in the outer fjords in Greenland. These discrepancies may be related to  
392 differences in sampling period (June or July), latitude (77.5°N vs 64-65°N) and currents  
393 in each fjord. In the present study, the slope of the NBSS of group C ( $-0.915 \pm 0.368$ )  
394 was steeper than those of the other groups ( $-0.745 \pm 0.434$ ) (Fig. 8 and Table 1). It  
395 suggests that high productivity occurred in the middle of the fjord. The steeper NBSS  
396 slope of group C would be caused by the dominance of small-sized cypris larvae. Since  
397 meroplanktonic larval phase of barnacles is limited for 2-3 weeks (Herz, 1933), these  
398 steeper NBSS slopes of group C in the middle of the fjord would be moderate, and the  
399 productivity would decrease after one month of this study.

400 For the inner fjords near glaciers in Greenland, the dominance of the copepods  
401 *M. longa* and *Pseudocalanus* spp. has been reported (Swalethorp et al., 2015). This may  
402 due to high biomass of their prey: i.e., protozooplankton, rotifer and copepod nauplii are  
403 available there, and it implies favourable food conditions for copepods (Calbet et al.,  
404 2011; Riisgaard et al., 2014; Swalethorp et al., 2015). Since there is a high gradient of  
405 suspended particulate matter in inner fjords, species-specific differences in tolerance for  
406 high sediment loads may explain their distribution (Arendt et al., 2011). For the glacial

407 fjord, subglacial discharge upwells and forms a sediment rich turbid meltwater plume  
408 (Chu, 2014). In the plume, high concentrations of suspended materials may affect  
409 feeding, egestion and reproduction of copepods. The ability to tolerate sediment is  
410 likely high for *M. longa* and low for *Calanus* spp., which determine their horizontal  
411 distribution: i.e., *M. longa* and *Calanus* spp. occurred in the inner fjords and outer ocean,  
412 respectively (Arendt et al., 2011).

413 In glacial fjords, the input of glacial meltwater provides nutrients and induces  
414 high primary production in the inner parts of the fjord (Arendt et al., 2010). In our study  
415 region of Bowdoin Fjord, tidewater glaciers discharge turbid subglacial freshwater into  
416 fjords, forming a plume and providing macronutrient by upwelling near the calving front  
417 (Kanna et al., 2018). Through enhanced primary production, high productivity of  
418 zooplankton is expected in front of the glacier. However, the slope of NBSS for  
419 zooplankton group B, which was observed near the glacier, did not vary with the other  
420 groups ( $p=0.769$ , one-way ANOVA). This indicates moderate zooplankton productivity  
421 there. Concerning taxonomic composition, the zooplankton community of group B  
422 contained a high composition of *Calanus* spp. and chaetognaths, instead of the reported  
423 species/taxa (e.g., *M. longa* and *Pseudocalanus* spp.). Since both *Calanus* spp. and  
424 chaetognaths are characterized with oceanic species (Arendt et al., 2010), the occurrence  
425 of them near the calving front in this study suggests that there was inflow from oceanic  
426 water through the bottom of the fjord and upwelling by plume in front of the glacier. In  
427 fact, upwelling of deep water in front of glacier has been reported for Bowdoin Fjord  
428 (Kanna et al., 2018). Since the sediment tolerance of *Calanus* spp. is low (Arendt et al.,  
429 2011), it may be hard for them to live in the inner fjord. Due to the large body size and  
430 high nutrition, the carcasses of *Calanus* spp. and chaetognaths would be a good food

431 sources for fishes (Arctic cod) and surface feeding sea birds (Black-legged Kittiwake  
432 *Rissa tridactyla*, Glaucous Gull *Larus hyperboreus* and Northern Fulmar *Fulmarus*  
433 *glacialis*), which form massive aggregations at the calving front of the Bowdoin Fjord  
434 (Nishizawa et al., submitted).

## 435 **5. Conclusions**

436 Based on zooplankton samples collected at the surface of Bowdoin Fjord in north-western  
437 Greenland, the zooplankton community structure was evaluated using three methods: a  
438 microscope, OPC and ZooScan. Among these methods, the analysis by ZooScan was  
439 able to filter out abiotic particles. Because of this advantage, it was shown that ZooScan  
440 provides more accurate abundance, biomass, size composition and NBSS data than the  
441 previously applied microscopic analysis, direct wet mass measurements and OPC  
442 measurements. Through analyses, the zooplankton community was clustered into three  
443 groups that characterized differences in dominant taxa. The horizontal distribution of  
444 the three groups clearly separated each other. The outer groups were dominated by  
445 jellyfishes, the middle fjord group was dominated by cypris larvae of barnacles, and the  
446 inner groups was characterized by large-sized *Calanus* spp. and chaetognaths. The  
447 large-sized zooplankton of the inner group suggests that they were transported from the  
448 outer fjord through layers of bottom water, then upwelled by the plume near the calving  
449 glacier. Since the large zooplankton contain more nutrition than the zooplankton of the  
450 other two groups, the inner fjord near the calving front would be a good feeding ground  
451 for fish and sea-birds.

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596 **Figure/table captions**

597 Fig. 1. Sampling location (a) and latitudinal changes in hydrography (temperature and  
598 salinity) (b) in Bowdoin Fjord during 27-29 July 2016. Open symbols:  
599 plankton sampling, dotted symbols: CTD measurement.

600 Fig. 2. Linear regressions of abundance (a) and biomass (b) of the total zooplankton  
601 community between different quantitative methods: microscopic count, direct  
602 wet mass (WM) measurement, OPC and ZooScan measurements. Each  
603 regression indicates the linear fit between one method (Y-axis) versus another  
604 method (X-axis). Factor means  $Y:X$ .

605 Fig. 3. Results of a cluster analysis based on zooplankton biomass derived from wet mass  
606 measurement (a). Three groups (A-C) and two out groups (Out) were identified  
607 at 47.5% similarity. The horizontal distribution of each group identified from  
608 cluster analysis on zooplankton biomass in the Bowdoin Fjord during 27– 29 July  
609 2016 (b).

610 Fig. 4. Comparison of abundance, biomass and taxonomic composition, which was  
611 quantified by microscopic count and direct measurements (left), and those by  
612 ZooScan measurements (right). Labels (Group A-C) below station numbers  
613 indicate clustered groups (cf. Fig. 3a).

614 Fig. 5. Relationships between ZooScan (Y-axis) and direct quantification (X-axis) on the  
615 abundance and biomass of each zooplankton species/taxon. Solid and dashed  
616 lines indicate 1:1 and significant relationships between the values, respectively.

617 Fig. 6. Comparison of abundance, biomass and size composition, which were quantified  
618 by OPC (left) and ZooScan (right). Sizes were arranged with five ESD size  
619 classes between 0.335-5 mm (0.335-1, 1-2, 2-3, 3-4, 4-5 mm). Labels (Group

620 A-C) below station numbers indicate clustered groups (cf. Fig. 3a).

621 Fig. 7. Relationships between abundance (left) and biomass (right) derived from ZooScan  
622 (Y-axis) and OPC (X-axis) of each zooplankton ESD size class (0.335-1, 1-2, 2-  
623 3, 3-4 and 4-5 mm). Solid and dashed lines indicate 1:1 and significant  
624 relationship between the values, respectively.

625 Fig. 8. NBSS for zooplankton at each station in the Bowdoin Fjord during 27-29 July  
626 2016. Open and solid symbols are the plots derived from OPC and ZooScan,  
627 respectively. Dashed and solid lines are the fitted plots of NBSS for OPC and  
628 ZooScan, respectively.

629 Table 1. List of NBSS slope and intercept derived from OPC and ZooScan at each station  
630 in the Bowdoin Fjord during 27-29 July in 2016.

631 Table 2. Results of ANCOVA for the slope of NBSS, with the intercept of NBSS and  
632 differences in instrument (i.e., OPC or ZooScan) applied as independent  
633 variables.

634

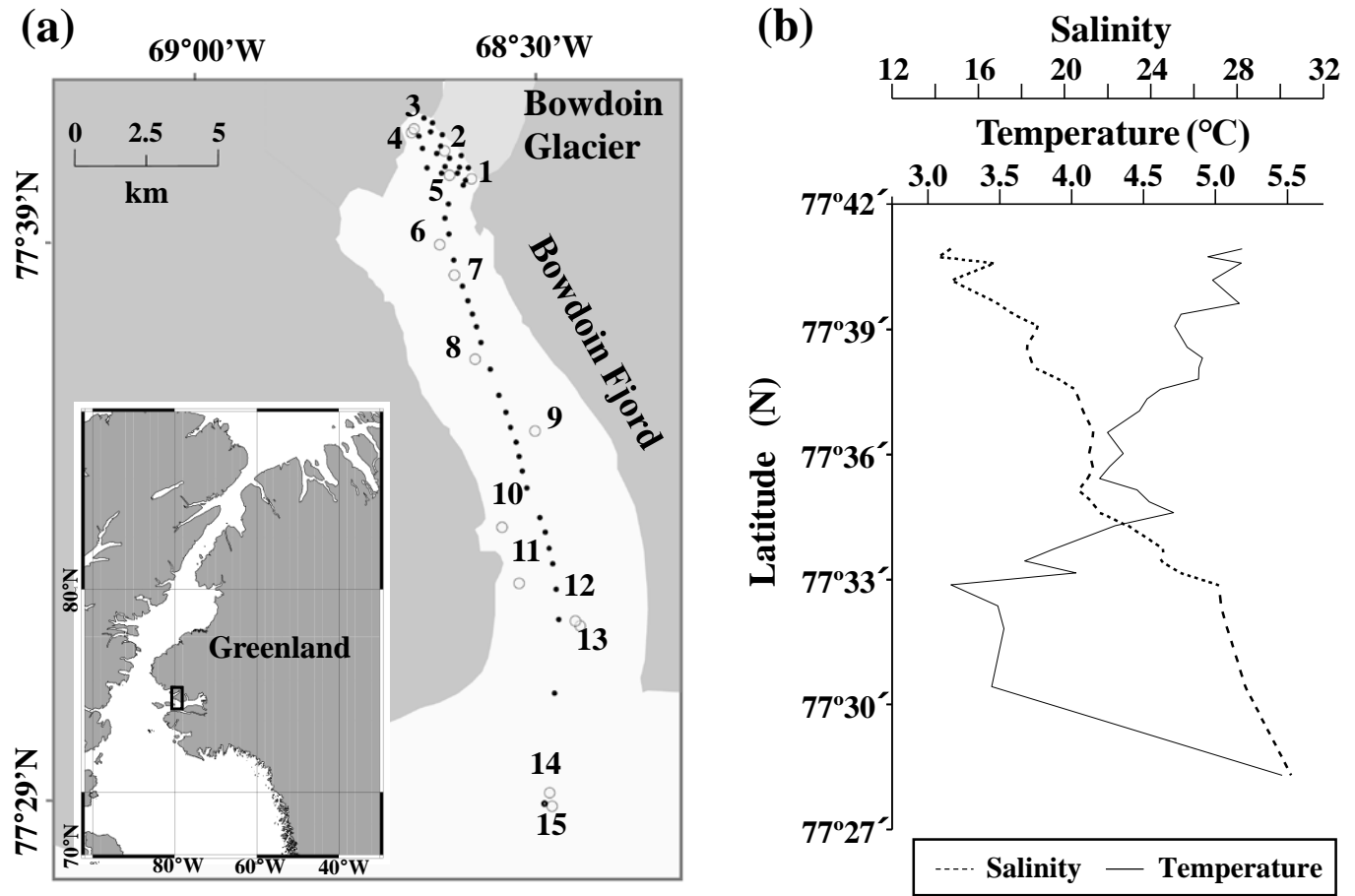


Fig. 1. Sampling location (a) and latitudinal changes in hydrography (temperature and salinity) (b) in Bowdoin Fjord during 27-29 July 2016. Open symbols: plankton sampling, dotted symbols: CTD measurement.

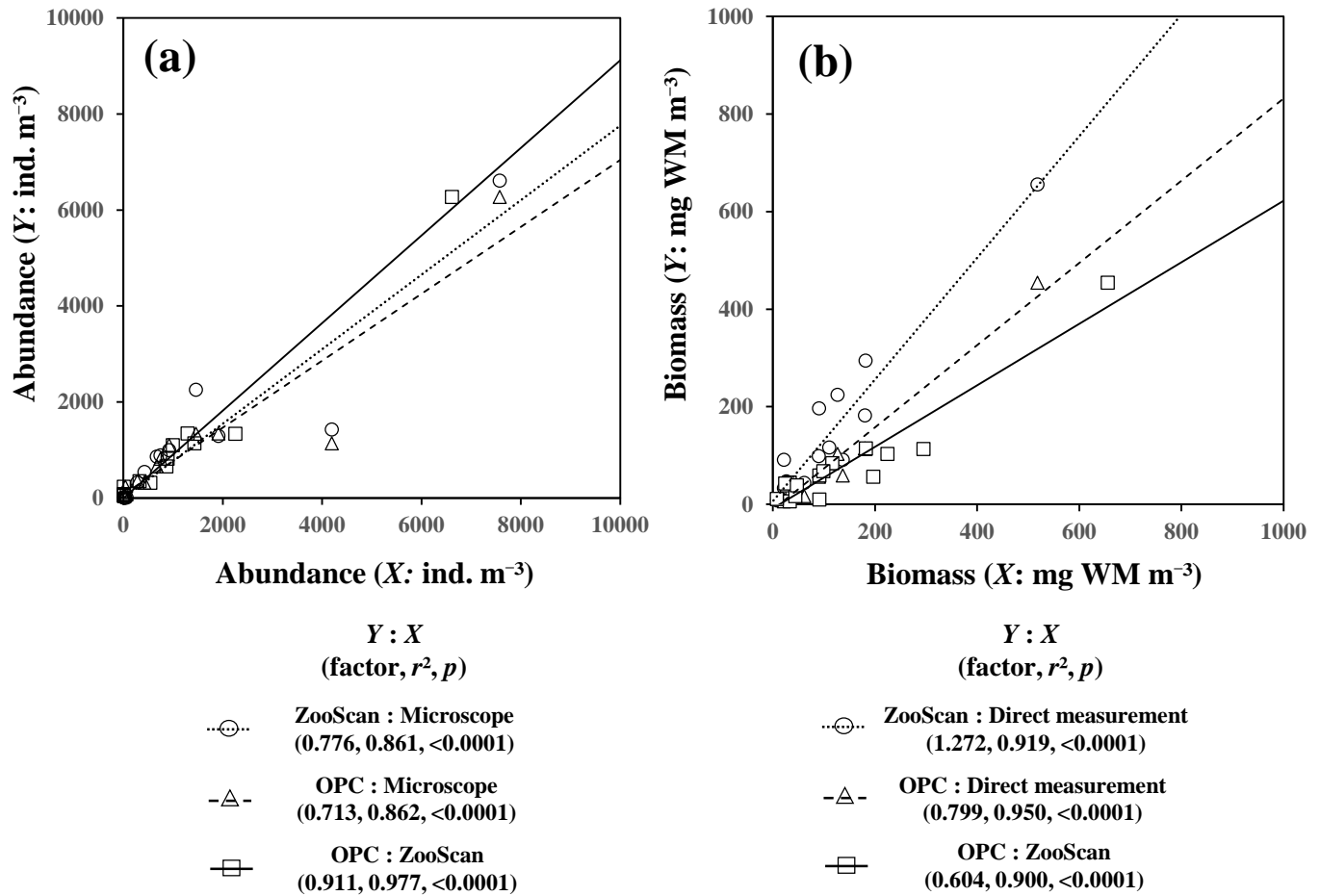


Fig. 2. Linear regressions of abundance (a) and biomass (b) of the total zooplankton community between different quantitative methods: microscopic count, direct wet mass (WM) measurement, OPC and ZooScan measurements. Each regression indicates the linear fit between one method (Y-axis) versus another method (X-axis). Factor means Y:X.



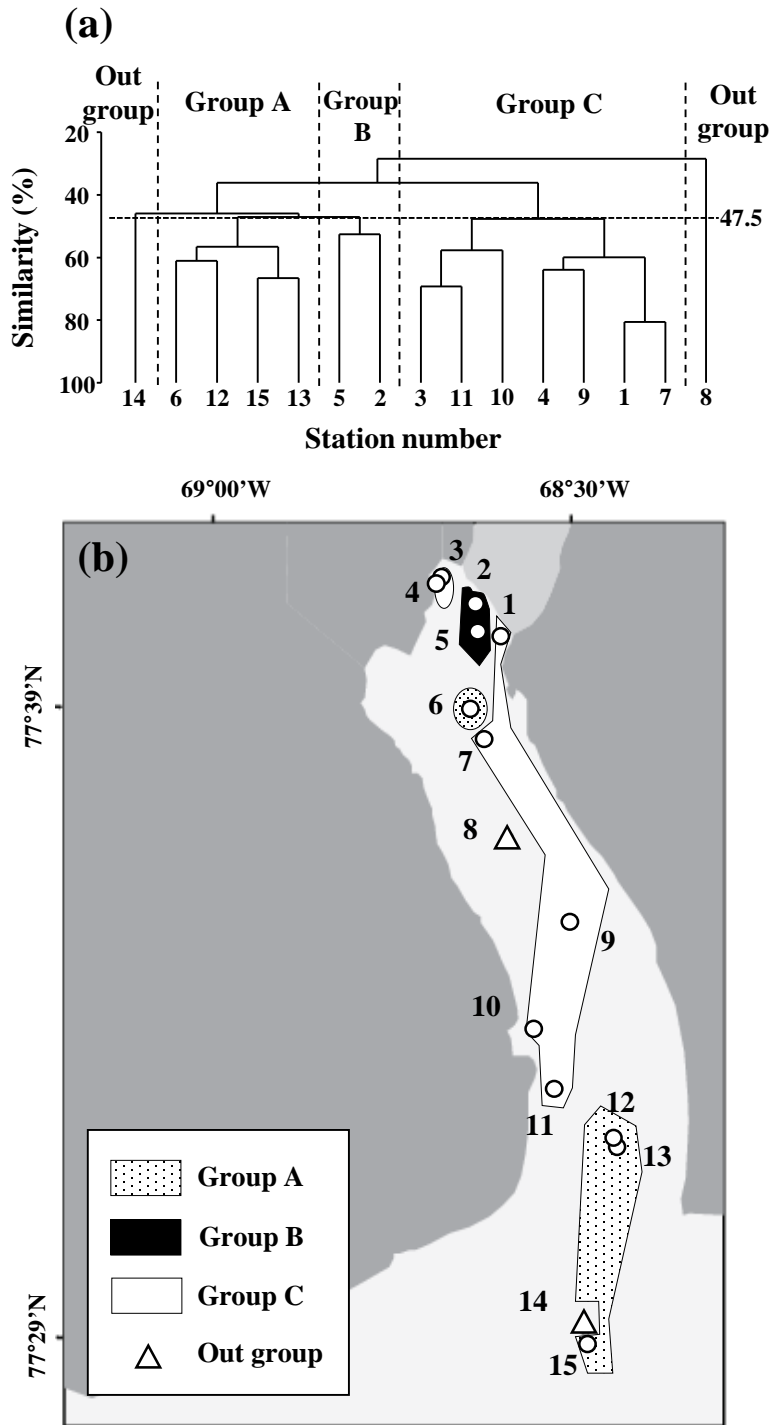


Fig. 3. Results of a cluster analysis based on zooplankton biomass derived from wet mass measurement (a). Three groups (A-C) and two out groups (Out) were identified at 47.5% similarity. The horizontal distribution of each group identified from cluster analysis on zooplankton biomass in the Bowdoin Fjord during 27–29 July 2016 (b).

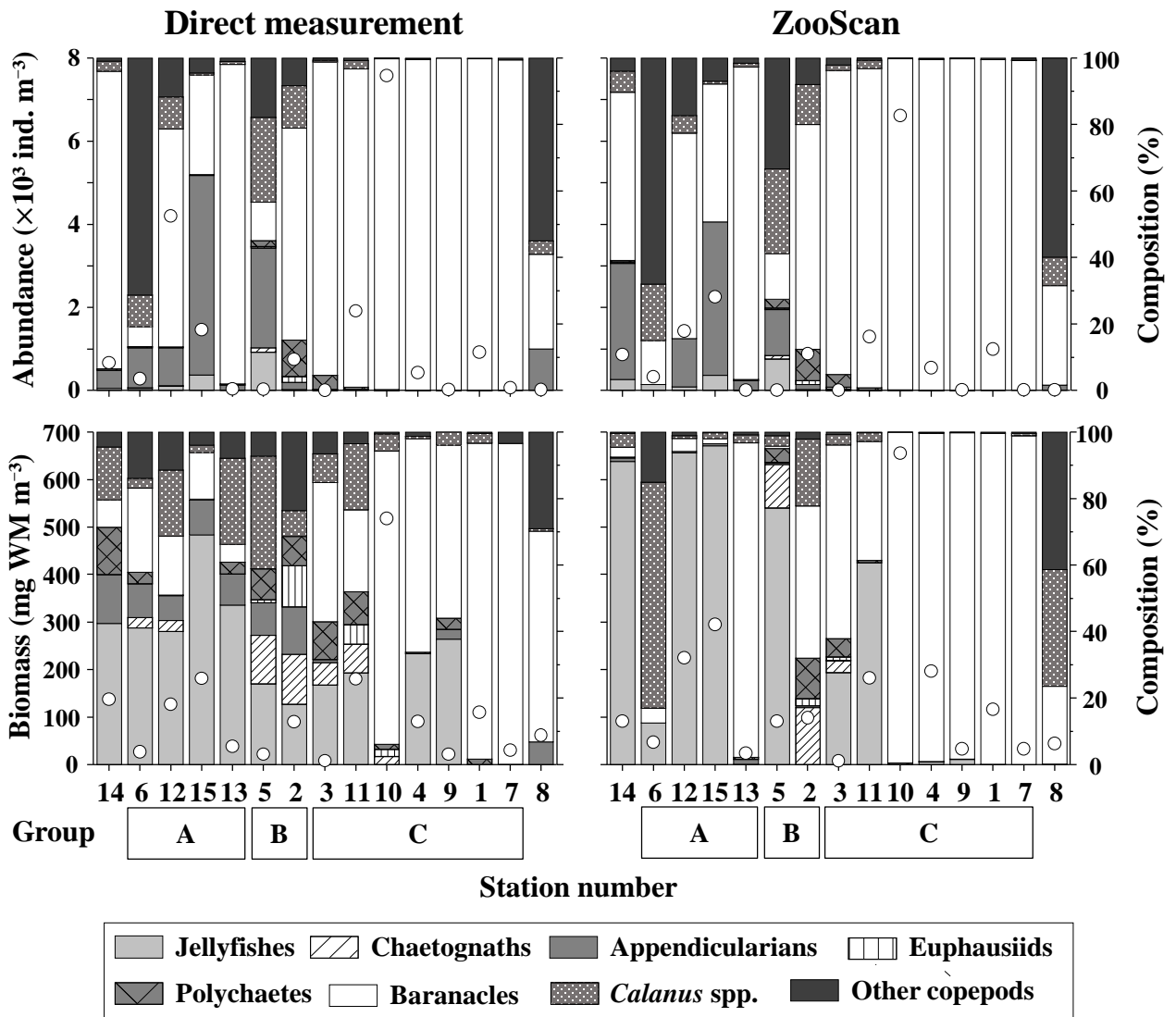


Fig. 4. Comparison of abundance, biomass and taxonomic composition, which was quantified by microscopic count and direct measurements (left), and those by ZooScan measurements (right). Labels (Group A-C) below station numbers indicate clustered groups (cf. Fig. 3a).

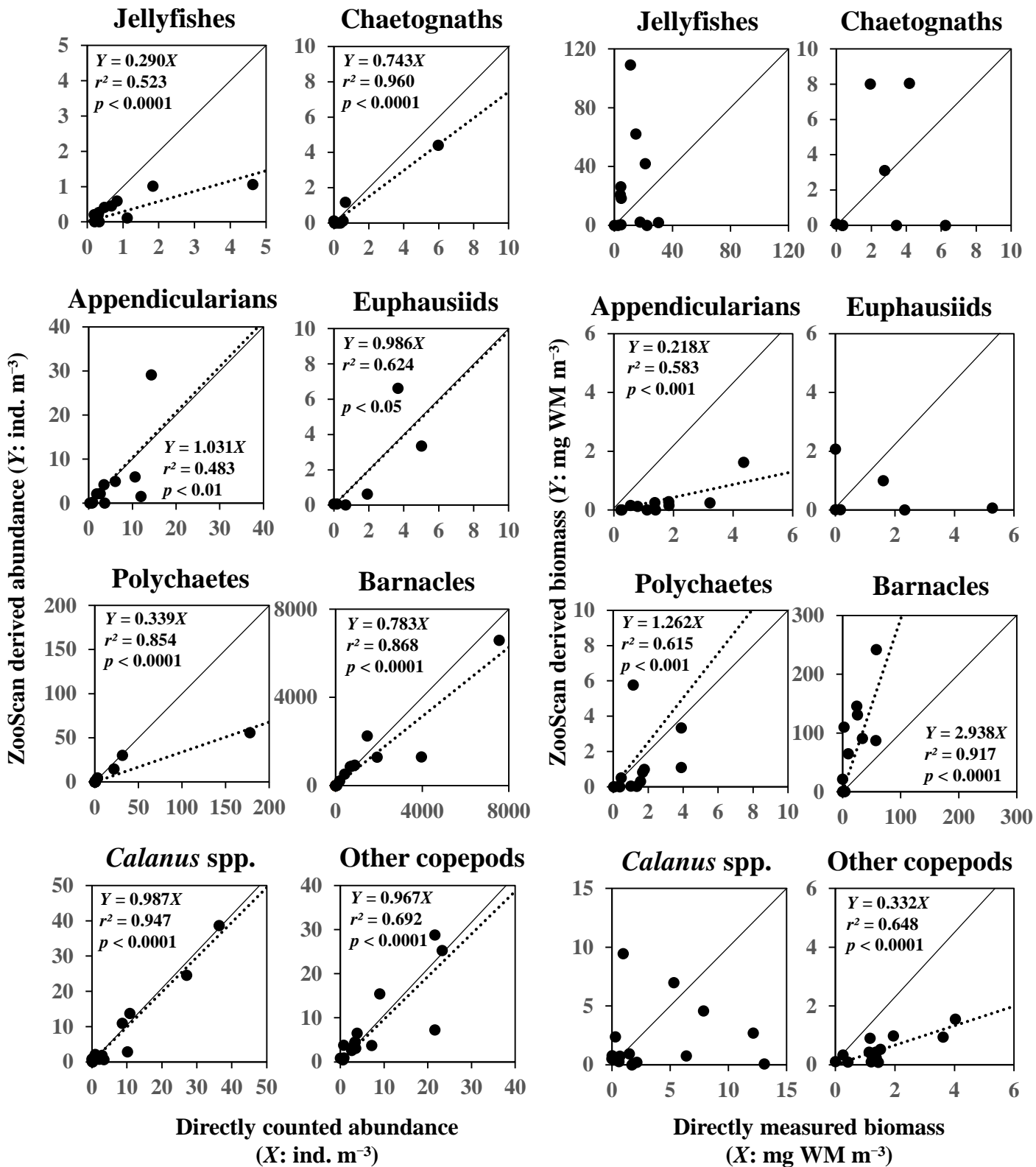


Fig. 5. Relationships between ZooScan ( $Y$ -axis) and direct quantification ( $X$ -axis) on the abundance and biomass of each zooplankton species/taxon. Solid and dashed lines indicate 1:1 and significant relationships between the values, respectively.

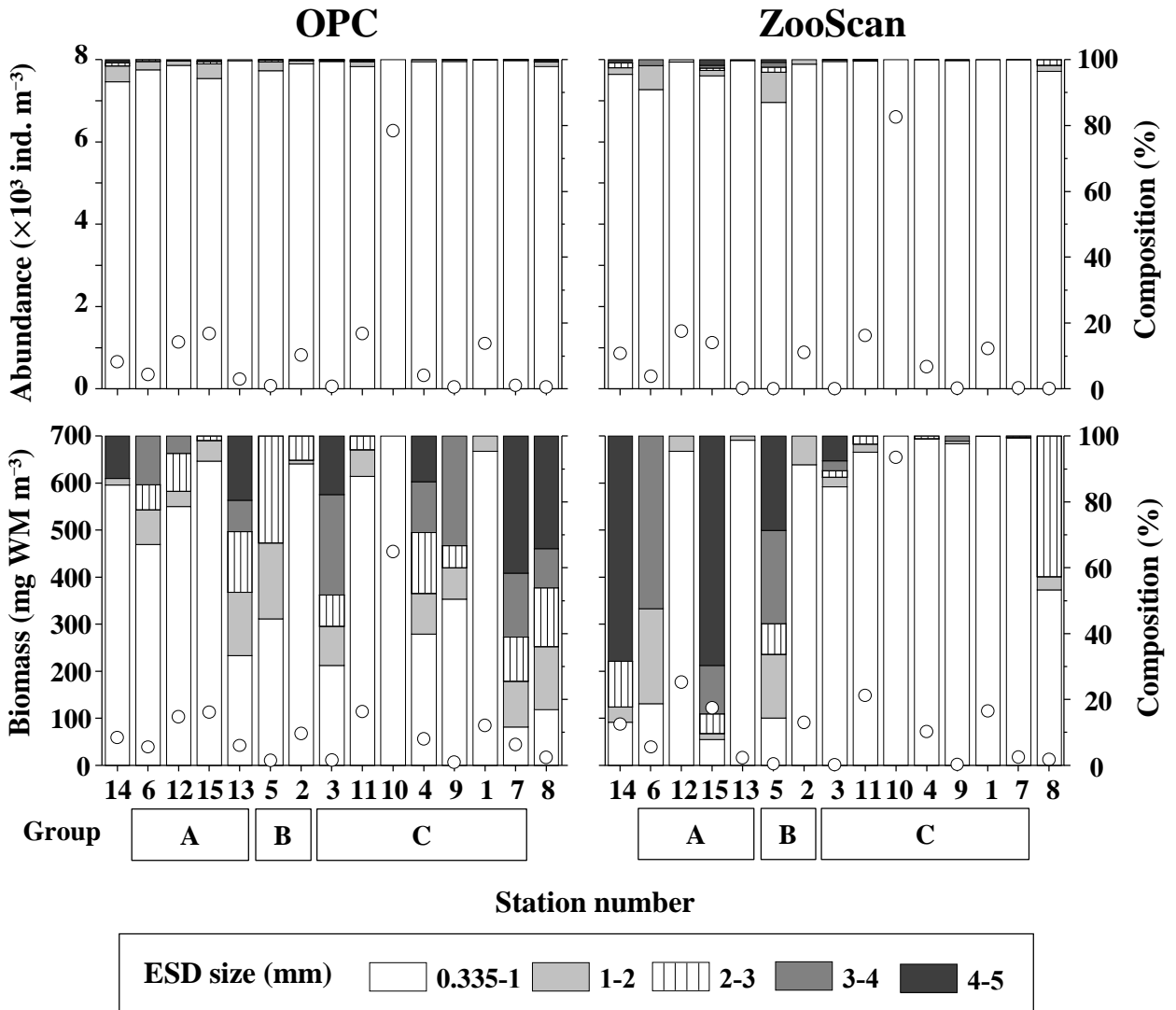


Fig. 6. Comparison of abundance, biomass and size composition, which were quantified by OPC (left) and ZooScan (right). Sizes were arranged with five ESD size classes between 0.335-5 mm (0.335-1, 1-2, 2-3, 3-4, 4-5 mm). Labels (Group A-C) below station numbers indicate clustered groups (cf. Fig. 3a).

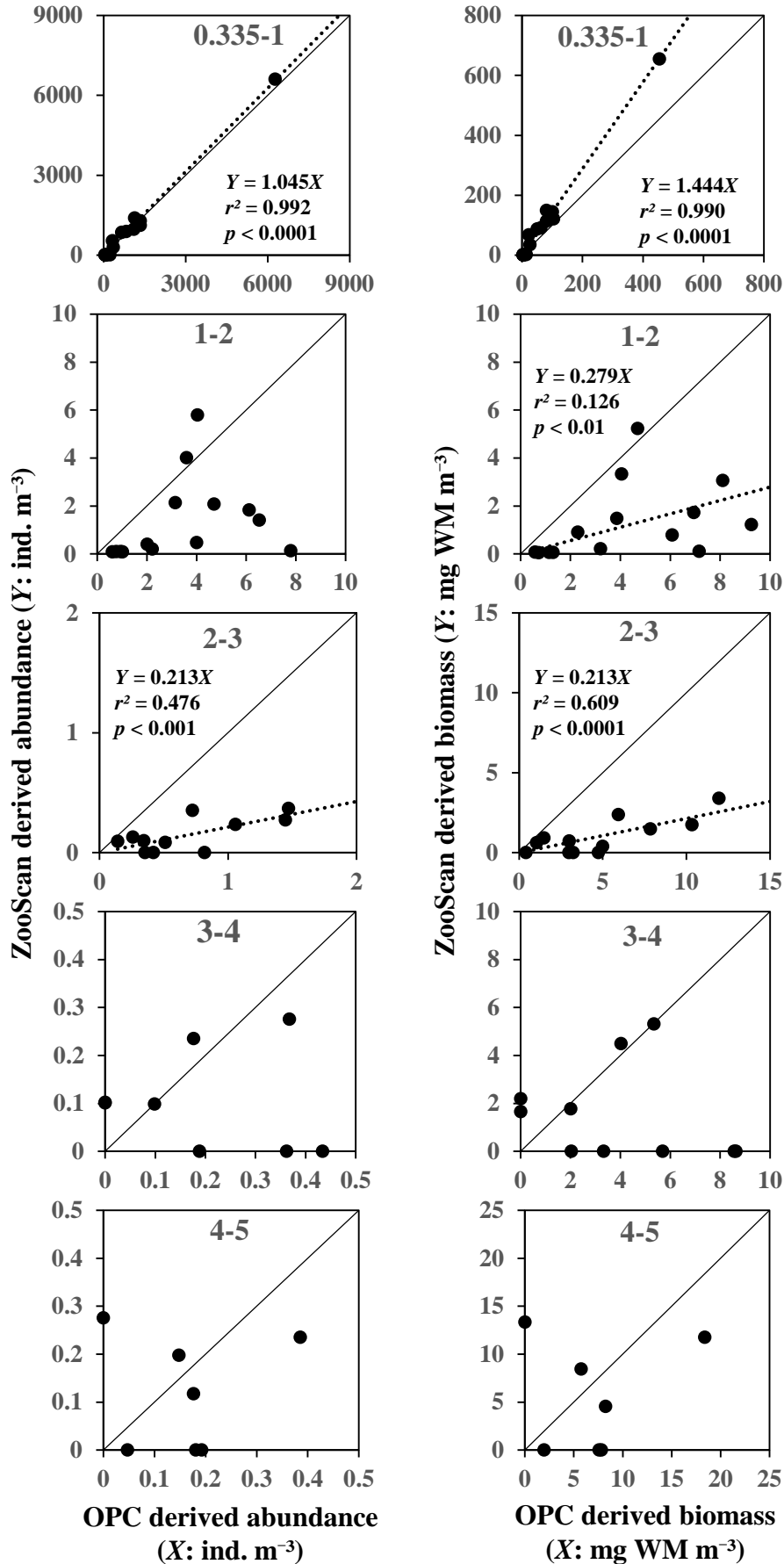


Fig. 7. Relationships between abundance (left) and biomass (right) derived from ZooScan (Y-axis) and OPC (X-axis) of each zooplankton ESD size class (0.335-1, 1-2, 2-3, 3-4 and 4-5 mm). Solid and dashed lines indicate 1:1 and significant relationship between the values, respectively.

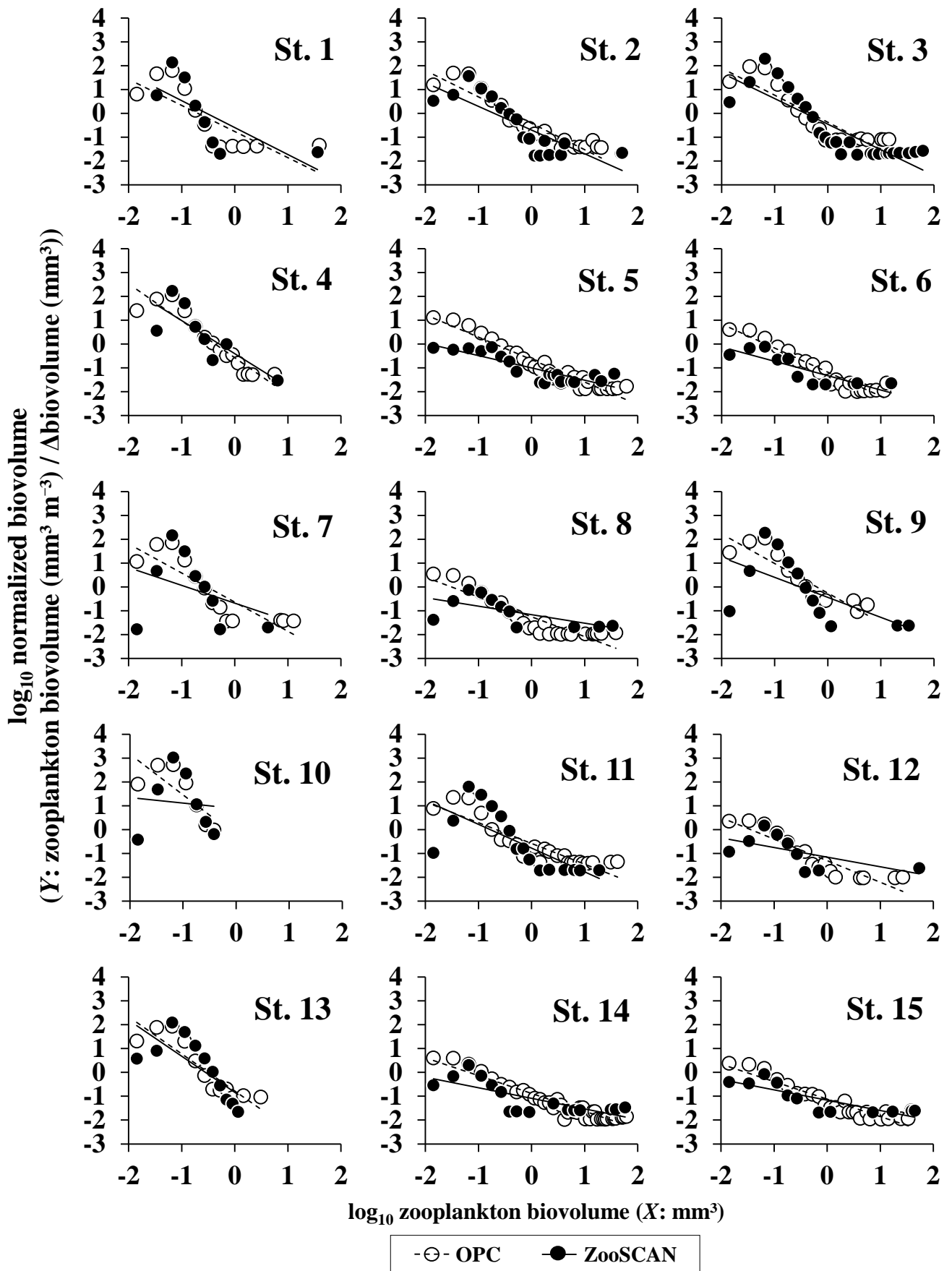


Fig. 8. NBSS for zooplankton at each station in the Bowdoin Fjord during 27-29 July 2016. Open and solid symbols are the plots derived from OPC and ZooScan, respectively. Dashed and solid lines are the fitted plots of NBSS for OPC and ZooScan, respectively.

Table 1. List of NBSS slope and intercept derived from OPC and ZooScan at each station in the Bowdoin Fjord during 27-29 July in 2016.

Station	NBSS derived from OPC			NBSS derived from ZooScan		
	Slope	Intercept	$r^2$	Slope	Intercept	$r^2$
1	-1.096	-0.762	0.608	-1.130	-0.597	0.520
2	-1.110	-0.415	0.877	-0.995	-0.697	0.686
3	-1.136	-0.368	0.806	-1.077	-0.451	0.739
4	-1.551	-0.578	0.882	-1.390	-0.407	0.650
5	-0.942	-0.656	0.931	-0.516	-1.005	0.732
6	-1.012	-1.179	0.915	-0.602	-1.326	0.720
7	-1.222	-0.639	0.751	-0.744	-0.685	0.137
8	-0.828	-1.258	0.832	-0.358	-1.168	0.427
9	-1.242	-0.262	0.835	-0.839	-0.443	0.385
10	-1.705	-0.245	0.607	-0.229	0.889	0.008
11	-0.868	-0.581	0.809	-0.997	-0.774	0.515
12	-0.905	-1.306	0.850	-0.406	-1.154	0.361
13	-1.562	-0.784	0.816	-1.516	-0.836	0.561
14	-0.747	-0.884	0.908	-0.446	-1.088	0.565
15	-0.737	-1.121	0.866	-0.433	-1.164	0.715

Table 2. Results of ANCOVA for the slope of NBSS, with the intercept of NBSS and differences in instrument (i.e., OPC or ZooScan) applied as independent variables.

Parameter	d.f.	SS	<i>F</i> -value	<i>p</i> -value
Intercept	1	0.304	2.423	0.1316
Instrument	1	0.304	6.538	0.0167
Instrument×Intercept	1	0.311	2.477	0.1276