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Causes of under- or overestimation of zooplankton biomass using Optical Plankton Counter (OPC): effect of size and taxa

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Abstract: Size-fractionated (0.25, 0.5, 1, 2, and 4 mm mesh size) wet mass (WM) and dry mass (DM) determinations and optical plankton counter (OPC) measurements were carried out on zooplankton samples collected at 15 stations in the northern North Pacific Ocean, Bering Sea and Chukchi Sea during July–August 2007. The total sample WM and DM estimated from OPC data corresponded closely to those of measured values by a factor of 0.970–1.098. However when the sample was portioned into different size groups, estimates of size-fractionated WM and DM by OPC data varied from measured masses by a factor of between 0.202 and 1.768. The high variabiliy was caused by an underestimation of sizes of the large sized (> 4 mm) fraction, or an overestimation of the number of the small size fraction (2–4 mm). The underestimation in the > 4 mm and overestimation in the 2–4 mm respectively were caused by the dominance of transparent hydromedusae, and slender-shaped euphausiids in the > 4 mm fraction. This study suggests that OPC analysis could be susceptible to errors in zooplankton biomass estimates in the large size fraction (> 4 mm) especially when euphausiids and hydromedusae dominate the population. On the other hand, OPC based estimates of DM within 0.25–4 mm size fraction are more robust, which may be due to the dominance of large copepods, and low detritus content in the samples from the oceanic subarctic Pacific, in summer 2007.

Key words: biomass estimation, optical plankton counter, size fraction, taxa

Introduction

In marine ecosystems a large proportion of the vertical material flux is due to meso- and macro-sized zooplankton (cf. Longhurst & Harrison 1989). In order to evaluate active and passive zooplankton flux, quantitative estimates of zooplankton biomass are of prime importance. Zooplankton biomass has been measured using various methods: that include the plankton net, coulter counter (Maddux & Kanwisher 1965), optical plankton counter (Herman 1988), video plankton recorder (Davis et al. 1992), and acoustics (Backus & Barnes 1957). Within these, the optical plankton counter (OPC) has been used in numerous studies (Sprules et al. 1998, Labat et al. 2002, Nogueira et al. 2004, Huntley et al. 2006), because it can measure rapidly and with ease, and can be used for in situ measurements also (Herman et al. 1993). The OPC provides particle count data in equivalent spherical diameter (ESD) size units. These sizes are proportional to the amount of light that is blocked by each organism as it passes through the OPC’s detector. An additional advantage of the OPC is that the detailed size data (4096 ESD size units between 0.25 mm and 20 mm in total) that become available can be readily applied for production estimation by empirical methods (cf. Ikeda 1985, Hirst et al. 2003).

The OPC technique has some shortcomings. Since it measures the size of particles based on the extent of attenuation of a light beam, the measurements could be impacted by a number of causes during the course of analysis. For instance, coincident counts (i.e. two or more particles coincident in the light beam will result in a single count and a size measurement equal to the sum of each size), particle shapes (e.g. slender) and the degree of particle transparency could cause underestimates in biomass and particle numbers (Herman 1992, Sprules et al. 1998, Zhang et al. 2000). Overestimation can result from fragmentation of zooplankton during the measurements, which in turn could lead to...
an overestimate in smaller size particles (Sprules et al. 1998, Beaulieu et al. 1999). There are times when non-zooplankton particles, such as during high detritus loads in the samples, could cause the OPC to provide inflated counts of zooplankton (Sprules 1998, Zhang et al. 2000).

Since the OPC can measure zooplankton abundance and biovolume of each size class, conversions of OPC biovolume data to wet mass (WM) or dry mass (DM), can be done with just one or two conversion factors for broader (0.25–20 mm) size ranges (cf. Sprules et al. 1998, Labat et al. 2002, Pollard et al. 2002, Nogueira et al. 2004, Huntley et al. 2006). However, direct comparisons between size-fractionated zooplankton masses and OPC-derived data have not been well studied. The only example is the work of Huntley et al. (2006), who compared net-based estimates of zooplankton biomass in three size fractions (<0.5 mm, 0.5–1 mm and >1 mm) with those derived from OPC measurements. The authors concluded that OPC and net data agreed with respect to total abundance and size composition but the biomass values for the <1 mm size fraction were higher in comparison with net-based biomass estimates because of detritus present in the samples. The study of Huntley et al. (2006) is based on samples collected in the offshore waters of Hawaii. Although this information is important, no comparable information is available for other regions of the world.

In the present study, we compared net-based size-fractionated biomass (WM and DM) with OPC measurements on samples collected at 15 stations in the northern North Pacific, Bering Sea and Chukchi Sea between 3 July and 11 August 2007 (Fig. 1). Based on a comparison between measured and OPC-estimated biomasses, we provide details of size class and taxa that can introduce errors in zooplankton mass calculated using OPC data, and suggest improved factors of biomass estimation in this region/season.

**Materials and Methods**

**Size-fractionated zooplankton masses**

Zooplankton samplings were conducted on T.S. *Oshoro-Maru* at 15 stations in the North Pacific, Bering Sea and Chukchi Sea between 3 July and 11 August 2007 (Fig. 1). Zooplankton samples were collected at night by vertical tows with a ring net (mouth opening 80 cm, mesh size 0.33 mm) from 0–150 m (stations where the bottom was deeper than 150 m) or 5 m above the bottom (stations where the bottom was shallower than 150 m). The volume of water filtered through the net was estimated using a flow-meter mounted in the mouth of the net.

Zooplankton samples were split using a Motoda box splitter (Motoda 1959), and one aliquot was fixed with 5% buffered formalin immediately on board for analysis with an OPC in the land laboratory. The remaining aliquot was size fractionated by gently sieving through five nested stainless mesh screens (mesh size: 0.25, 0.5, 1, 2, and 4 mm) within a sea water-filled large bucket. Sizes of these five mesh screens were selected to obtain sufficient amounts of each size-fractionated sample to determine the zooplankton mass. After sieving, each fraction was filtered on pre-weighed nylon mesh (0.13 mm or 0.18 mm mesh size) under low vacuum and briefly rinsed with distilled water, packed in aluminum foil, placed into sealed plastic bags, and stored in a freezer (−30°C). During processing of the samples, the dominant taxa in terms of wet mass were recorded for each size class. In the shore laboratory, frozen samples were weighed for wet mass (WM) with a precision of 1 mg using an electronic balance (Sartorius 2001 MP2), and freeze-dried to determine the dry mass (DM).

**OPC analysis of zooplankton samples**

Formalin-preserved zooplankton samples were used for measurements with an OPC. Measurements were made with a bench-top OPC (Model OPC-1L: Focal Technologies Corp.) using 1/2–1/16 subsamples (varied according to the amount of the samples) of the total formalin preserved samples. For avoiding coincidence and detritus detection, the measurements were done at a flow rate of 10 L min⁻¹ and particle density of <10 counts sec⁻¹, respectively. To avoid fragmentation of the zooplankton, the samples were analyzed once.

Abundance per square meter (*N*, indiv. m⁻²) in each 4096 ESD size unit was calculated from the following equation:

\[
N = \frac{n \times d}{s \times F}
\]
where \( n \) is the number of particles (indiv.) counted, \( s \) is split ratio of each sample, \( F \) is filtered volume of the net (m\(^3\)), and \( d \) is the net towed depth (m).

Wet mass of the zooplankton community was calculated from the ESD data by assuming the relative density of zooplankton as equal to water (Gallienne et al. 2004, Liebig et al. 2006). WM was converted to DM (DM=0.1×WM), on the basis of the assumption that the water content of subarctic to subtropical zooplankton in the North Pacific Ocean above 1,000 m was 90% (Yamaguchi et al. 2005).

To enhance the accuracy of OPC-derived masses, we compared directly measured mass (WM and DM) in each size class (0.25–0.50, 0.5–1.0, 1–2, 2–4, >4 mm) with the OPC-estimated mass in the same ESD range (0.25–0.50, 0.5–1.0, 1–2, 2–4, >4 mm ESD). Simple correlation analyses were performed on data from each size class, and conversion factors were calculated when significant correlations were observed.

**Results**

Dominant species/taxa in plankton samples of each size class are shown in Table 1. For the 0.25–0.50 mm size class, adult and late copepodite stages of *Pseudocalanus* spp. dominated, while *Metridia pacifica* Brodsky or *Neocalanus plumchrus* Marukawa dominated for 0.5–1.0 mm and 1–2 mm, respectively (Table 1). For the large size class (>4 mm), hydromedusae (*Aglantha digitale* Müller) or furcilia of euphausiids (*Euphausia pacifica* Hansen or *Thysanoessa spp.*) were dominant in most of the samples, and their dominances were prominent especially at two stations (Stn 5 for *Thysanoessa longipes* Brandt and Stn 7 for *A. digitale*, Table 1). It should be noted that there were only small amounts of detrital materials in all of the samples (Table 1).

The size-fractionated WM and DM were compared with those estimated by the OPC data (Table 2). For the total fraction, OPC-derived WM was slightly higher (1.098×) than directly measured values, while the OPC-derived DM was slightly lower (0.970×) than directly measured values. The OPC-derived biomass in the largest (>4 mm) size class was significantly (\( t \)-test, \( p<0.01 \)) lower, both in WM (0.202×) and in DM (0.216×). In the 2–4 mm size class, the OPC-derived biomass was observed significantly higher both in WM (1.768×) and in DM (1.396×). The overestimation in OPC-derived biomass was also observed for WM in the 0.5–1.0 mm and 1–2 mm size classes (1.511× and 1.218×). For all size fractions, significant correlations between OPC-derived and directly measured masses were observed (\( p<0.05 \)), especially for the total size class (\( p<0.001 \)) (Table 2).

**Discussion**

OPC-derived biomass in the largest (>4 mm) size class was a highly significant underestimate (20–22%) of the directly measured value (Table 2). The scatter plot for the large size class (>4 mm) showed that the OPC-derived masses for most of the stations were lower for both WM and DM (Fig. 2). The stations where the OPC-derived masses were significant underestimates (shown with solid triangles and open diamond symbols in Fig. 2) were different for WM and DM. For WM, the OPC underestimation was large at Stn 7 (Fig. 2), where the hydromedusa *Aglantha digitale* predominated (Table 1). OPC underestimation of sizes of hydromedusae was reported by Beaulieu et al. (1999). Recently, Yokoi et al. (2008) reported that the sizes of gelatinous zooplankton such as doliolids and salps tended to be underestimated during OPC analysis because of their transparent and soft bodies which easily flatten out or fragment during analysis. While Yokoi et al. (2008) did not study hydromedusae, the underestimation of the size of these transparent gelatinous zooplankton, could have been the likely cause of the OPC underestimating its WM. Since hydromedusae have a high water content (99%, cf. Postel et al. 2000), OPC size underestimates at this station may be prominent only for WM.

![Fig. 2. Relationship between OPC-derived and directly measured biomass (upper: wet mass, lower: dry mass) for large (>4 mm) sized zooplankton. The solid triangle and open diamond indicates five and seven stations, respectively. The solid lines indicate the regression lines between OPC-derived and directly measured biomass for total data (cf. Table 2). The dashed lines indicate the regression lines between OPC-derived and directly measured biomass after removing open diamond data in WM or solid triangle data in DM; see main text for explanation. The dotted lines indicate positions of 1 : 1. *: \( p<0.05 \), **: \( p<0.01 \).](image-url)
For DM, underestimation by the OPC was large at Stn 5 (Fig. 2), where the euphausiid *Thysanoessa longipes* predominated (Table 1). Euphausiid sizes are known to be underestimated during OPC analysis, because of their slender body shape (Herman 1992). The slender body shape of euphausiids induces underestimation of size when the specimen passing through the OPC detector is parallel to the light beam (Herman 1992). Since euphausiids have a low water content (ca. 80%, cf. Mauchline 1980), its impact on OPC size underestimations at this station may not be significant for WM, but for DM only.

In the 2–4 mm size class (Table 2), two causes need to be taken into account to explain the overestimates by the OPC. One is the size underestimation of large particles by the OPC and the other is the coincident detection of smaller (<2 mm size class particles) and larger (size overestimation of small particles). In the present study, we believe that the OPC overestimation in the 2–4 mm size class is caused by the underestimation of the plankton size of the large >4 mm size fraction. As mentioned before, this underestimation of plankton size in large size can easily occur due to transparency (as in hydromedusae) or slenderness (as in euphausiids). The species/taxa abbreviations are following, *Aglantha digitale* Müller: Ad; *Calanus marshallae* Frost: Cm; Chaetognaths: Ch; *Clione* sp.: Cl; *Ctenophore*: Ct; *Cyphocaris* sp.: Cy; *Euphausia pacifica* Hansen: Ep; *Euphausiids*: Eu; *Hydromedusae*: Hy; *Limacina* sp.: Li; *Metridia pacifica* Brodsky: Mp; *Neocalanus cristatus* Kröyer: Nc; *Neocalanus plumchrus* Marukawa: Np; *Oikopleura* spp.: Oi; *Phytoplankton*: Ph; *Pseudocalanus* spp.: Ps; *Themisto* spp.: The; *Thysanoessa longipes* Brandt: Tl; *Thysanoessa* spp.: Thy; copepods (co), adults (ad), furcilia (fu).

### Table 1. Dominant species/taxa in each size class of the plankton samples collected from fifteen stations in the northern North Pacific, Bering Sea and Chukchi Sea during July–August 2007. For the location of each station, see Fig. 1. The predominant component, species/taxa are underlined. Species/taxa abbreviations are following, *Aglantha digitale* Müller: Ad; *Calanus marshallae* Frost: Cm; Chaetognaths: Ch; *Clione* sp.: Cl; *Ctenophore*: Ct; *Cyphocaris* sp.: Cy; *Euphausia pacifica* Hansen: Ep; *Euphausiids*: Eu; *Hydromedusae*: Hy; *Limacina* sp.: Li; *Metridia pacifica* Brodsky: Mp; *Neocalanus cristatus* Kröyer: Nc; *Neocalanus plumchrus* Marukawa: Np; *Oikopleura* spp.: Oi; *Phytoplankton*: Ph; *Pseudocalanus* spp.: Ps; *Themisto* spp.: The; *Thysanoessa longipes* Brandt: Tl; *Thysanoessa* spp.: Thy; copepods (co), adults (ad), furcilia (fu).

<table>
<thead>
<tr>
<th>Station No.</th>
<th>Size class (mm)</th>
<th>0.25–0.50</th>
<th>0.5–1.0</th>
<th>1–2</th>
<th>2–4</th>
<th>&gt;4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ps (co and ad)</td>
<td>Mt (co and ad)</td>
<td>Mt (co and ad), small The</td>
<td>Mt (co and ad), small The</td>
<td>Mt (co and ad), small The</td>
<td>Mt (co and ad), small The</td>
</tr>
<tr>
<td>2</td>
<td>No dominant taxa</td>
<td>Mt (co and ad), small The</td>
<td>Mt (co and ad), small The</td>
<td>Mt (co and ad), small The</td>
<td>Mt (co and ad), small The</td>
<td>Mt (co and ad), small The</td>
</tr>
<tr>
<td>3</td>
<td>Ps (co and ad)</td>
<td>Mt (co and ad)</td>
<td>Mt (co and ad)</td>
<td>Mt (co and ad)</td>
<td>Mt (co and ad)</td>
<td>Mt (co and ad)</td>
</tr>
<tr>
<td>4</td>
<td>Ps (co and ad)</td>
<td>Mt (co and ad)</td>
<td>Mt (co and ad)</td>
<td>Mt (co and ad)</td>
<td>Mt (co and ad)</td>
<td>Mt (co and ad)</td>
</tr>
<tr>
<td>5</td>
<td>Ph</td>
<td>Mt (co and ad), small The</td>
<td>Mt (co and ad), small The</td>
<td>Mt (co and ad), small The</td>
<td>Mt (co and ad), small The</td>
<td>Mt (co and ad), small The</td>
</tr>
<tr>
<td>6</td>
<td>No dominant taxa</td>
<td>Mt (co and ad), small The</td>
<td>Mt (co and ad), small The</td>
<td>Mt (co and ad), small The</td>
<td>Mt (co and ad), small The</td>
<td>Mt (co and ad), small The</td>
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<tr>
<td>7</td>
<td>Ps (co and ad)</td>
<td>Mt (co and ad)</td>
<td>Mt (co and ad)</td>
<td>Mt (co and ad)</td>
<td>Mt (co and ad)</td>
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<tr>
<td>11</td>
<td>Ps (co and ad)</td>
<td>Small Hy</td>
<td>Small Hy</td>
<td>Small Hy</td>
<td>Small Hy</td>
<td>Small Hy</td>
</tr>
<tr>
<td>12</td>
<td>Ph, Ps (co and ad)</td>
<td>Small Hy</td>
<td>Small Hy</td>
<td>Small Hy</td>
<td>Small Hy</td>
<td>Small Hy</td>
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<tr>
<td>13</td>
<td>No dominant taxa</td>
<td>Mt (co and ad), Oi</td>
<td>Mt (co and ad), Oi</td>
<td>Mt (co and ad), Oi</td>
<td>Mt (co and ad), Oi</td>
<td>Mt (co and ad), Oi</td>
</tr>
<tr>
<td>14</td>
<td>Ps (co and ad)</td>
<td>Small Eu (fu)</td>
<td>Small Eu (fu)</td>
<td>Small Eu (fu)</td>
<td>Small Eu (fu)</td>
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<td>15</td>
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<td>Mt (co and ad)</td>
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<td>Mt (co and ad)</td>
<td>Mt (co and ad)</td>
<td>Mt (co and ad)</td>
</tr>
</tbody>
</table>

### Table 2. Factor between OPC-derived and directly measured WM and DM (OPC: measured). *: p<0.05, **: p<0.01, ***: p<0.001.

<table>
<thead>
<tr>
<th>Unit</th>
<th>Size class</th>
<th>Factor</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>WM</td>
<td>Total</td>
<td>1.098</td>
<td>0.46***</td>
</tr>
<tr>
<td></td>
<td>&gt;4 mm</td>
<td>0.202</td>
<td>0.26**</td>
</tr>
<tr>
<td></td>
<td>2–4 mm</td>
<td>1.768</td>
<td>0.65***</td>
</tr>
<tr>
<td></td>
<td>1–2 mm</td>
<td>1.218</td>
<td>0.59***</td>
</tr>
<tr>
<td></td>
<td>0.5–1.0 mm</td>
<td>1.511</td>
<td>0.81***</td>
</tr>
<tr>
<td></td>
<td>0.25–0.50 mm</td>
<td>0.751</td>
<td>0.49**</td>
</tr>
<tr>
<td>DM</td>
<td>Total</td>
<td>0.970</td>
<td>0.54***</td>
</tr>
<tr>
<td></td>
<td>&gt;4 mm</td>
<td>0.216</td>
<td>0.09*</td>
</tr>
<tr>
<td></td>
<td>2–4 mm</td>
<td>1.396</td>
<td>0.68***</td>
</tr>
<tr>
<td></td>
<td>1–2 mm</td>
<td>0.860</td>
<td>0.30*</td>
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<tr>
<td></td>
<td>0.5–1.0 mm</td>
<td>0.861</td>
<td>0.30*</td>
</tr>
<tr>
<td></td>
<td>0.25–0.50 mm</td>
<td>0.522</td>
<td>0.30*</td>
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</table>
phausiids) (Fig. 2). Since hydromedusae or euphausiids were predominant in the large size fraction (>4 mm) at most of the stations (Table 1), severe mass underestimation by OPC in the large size fraction (>4 mm) occurred for both WM and DM (Table 2). The coincidence of particles in this study should have been extremely low because of the low particle density during OPC measurement. At high particle densities, the coincidence of some particles can occur during OPC analysis (Sprules 1998). If the OPC measured only the 0.5 mm ESD size (which is the size at abundance peak in most samples) spherical particles under the present study condition, values were 10 L min\(^{-1}\) and 10 counts sec\(^{-1}\). The probability of coincidence occurring was calculated from the following equation:

\[
C = \left(1 - \prod_{i=2}^{k} \left(1 - \frac{W - (i-1)B}{W+1}\right)\right) \times 100, \tag{2}
\]

where \(C\) is the probability (%) of coincidence occurring, \(W\) is the cross-sectional area of water passing through the OPC (= water volume through OPC during 1 second/17 mm beam length between the transmission window and the aperture within OPC, mm\(^2\)), \(B\) is the cross-sectional area of a spherical particle (mm\(^2\)), \(k\) is the number of same ESD size particles detected. The probability estimate was sufficiently low (0.089%). We believe that the coincidence of smaller size class particles was not the main reason for the overestimation.

The OPC underestimation in the large size fraction may vary with the location and season, thus the application of conversion factors in this study might be limited within this location and season. Nonetheless, our results are noteworthy, in the sense that they offer a reason for caution when reporting OPC-derived sizes. The degree of OPC underestimation in size, which was caused by transparency (gelatinous zooplankton) or slenderness (euphausiids and chaetognaths), is known to be greater for large sized zooplankton (cf. Fig. 1 of Yokoi et al. 2008).

In addition to this, differences in the units of zooplankton mass (WM or DM) implies that differences in taxa can lead to underestimates by the OPC, i.e. gelatinous zooplankton for WM, euphausiids for DM (Fig. 2). Differences between WM and DM were also evident in the 0.5–1.0 and 1–2 mm size range. OPC overestimation was seen in WM while it was not observed in DM (Table 2). Around the 0.5–2 mm size range, copepods were the dominant taxa, while gelatinous zooplankton (hydromedusae and Oikopleura spp.) were observed at several stations (Stn 11–13, Table 1). This implies that the effect of gelatinous zooplankton (causes of OPC overestimation in WM) was more serious down to the smaller size range.

In conclusion, while estimates of total mass by OPC data correspond closely with those of directly measured (0.970–1.098), estimates of zooplankton mass (WM and DM) by OPC data varied by a factor of 0.202–1.768 in comparison with measured values. The cause for this discrepancy in the latter is because of OPC underestimation of the large-sized fraction and overestimation of the small size fraction. The dominance of hydrozoa and euphausiids may imply underestimation of the masses in WM and DM, respectively (Fig. 2). Accurate zooplankton biomass estimates were possible for the 0.25–4 mm fraction especially for DM (Table 2). Such accurate estimates may be possible for samples which are copepod dominated and have low detrital content (Table 1) as is the case for the oceanic subarctic Pacific. Despite the discrepancy between values, we contend that the OPC is a powerful tool for zooplankton size, WM and DM estimates of preserved zooplankton samples and accurate estimates are possible if a priori knowledge of the taxonomic composition of the region is available.

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