

## The use of the Laser Optical Plankton Counter to measure zooplankton size, abundance, and biomass in small freshwater lakes

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### Abstract

The Optical Plankton Counter (OPC) has been used in a variety of environments since its introduction over decade ago, but its use in freshwater lakes has been limited by high densities of zooplankton and detritus. The newer Laser Optical Plankton Counter (LOPC) has several modifications from its predecessor, and the goal of this study was to examine whether it could be used to measure average size ( $\mu\text{m}$  equivalent spherical diameter, ESD), abundance (particles  $\text{L}^{-1}$ ), and biomass ( $\mu\text{g}$  dry weight  $\text{L}^{-1}$ ) of zooplankton in samples from 18 lakes in the Eastern Townships region of Quebec, Canada. The LOPC slightly overestimated the size of copepods, and consistently underestimated *Daphnia* by approximately 25% ESD. Densities and biomass of net samples were very similar between the LOPC lab version and traditional microscope analyses suggesting that the LOPC can be reliably used to process preserved net samples. When the LOPC was towed in situ vertically in Lake Memphremagog, QC, Canada, estimated zooplankton abundances were ten times net sample values from the same water column, but similar abundances were found between the LOPC and pumped zooplankton samples at 2 m depth. These results indicate that the LOPC may be well suited for analyses of zooplankton abundance and biomass in productive freshwater lakes.

The introduction of the Optical Plankton Counter provided the opportunity for quick and easy enumeration of zooplankton based on body size (Herman 1988, Herman 1992). It has been used in a variety of marine environments (Huntley et al. 1995, Grant et al. 2000, Rensen et al. 2004) and large inland lakes (Sprules et al. 1998, Yurista et al. 2005), but to date, the use of the OPC in small freshwater lakes has been limited. Gal et al. (1999) used the OPC to examine the distribution of large *Mysis relicta*, but did not examine the smaller, more numerous zooplankton. Patoine et al. (2002) used the OPC to evaluate size structure of preserved zooplankton samples in 38 lakes, and Patoine et al. (2006) compared biomass of preserved samples by traditional microscope and seston measurements to the lab version of the OPC. The use of optical plankton counters to measure in situ abundance and biomass of crustacean zooplankton has not yet been successful in small lakes.

Many difficulties arise when comparing OPC estimates of zooplankton abundance and biomass, in both marine and freshwater systems. OPC estimates have been shown to agree with (Ruberg and Eadie 2000), overestimate (Halliday et al. 2001), or underestimate (Herman 1988) zooplankton abundance and biomass compared with traditional net samples. Causes attributed to discrepancies between net and OPC densities are coincident counts, a lower detection limit of the OPC than the net, variation in particle orientation through the OPC light beam, clogging of the net, extrusion of zooplankton through the net, incorrect estimation of sample volume, the disparity of scales between sampling systems, and counting of large phytoplankton or phytoplankton aggregates (Nogueira et al. 2004). In productive freshwater systems, coincident counts (where two or more particles are counted together as one large particle) are considered to be particularly problematic and confound interpretation to the point where data obtained by using the OPC in situ would not be informative in these systems (Sprules et al. 1998).

The next generation of the OPC, the Laser OPC (Herman et al. 2004) or LOPC, may possess the necessary modifications to accurately enumerate zooplankton in lakes with high particle abundance and reduce coincident counts. Specifically, the LOPC is capable of distinguishing individual particles at abundances up to  $10^6 \text{ m}^{-3}$ , which is nearly 100 times higher than the abun-

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dance at which the OPC could differentiate individual particles. Additionally, the LOPC directly measures water flow rate through the machine, which is crucial for accurate sample volume estimates (Herman et al. 2004). Counts of large phytoplankton and/or detrital aggregates may still confound comparisons of zooplankton abundance and must be considered a possible source of discrepancies. This study will explore the application of the LOPC to measure the abundance and biomass of zooplankton in small lakes, where concentrations of animals tends to be higher than marine systems and the Great Lakes.

There are three main steps necessary to compare plankton net samples processed by microscope analyses with LOPC estimates: (1) determine the correlation between size of individual animals measured by the LOPC (estimated as equivalent spherical diameter, ESD) and traditional microscope measurements to determine which species of zooplankton are likely to be detected by the LOPC and to evaluate how different groups are counted by the LOPC; (2) determine how the LOPC benchtop setup (consisting of the LOPC connected to a circulating water flow, called the "lab circulator"), abundance, and biomass estimates compare with traditional microscope counts and calculated biomass (based on species length-weight regressions) of the same preserved net samples; and (3) determine the correlation between the LOPC towed in situ vertically in a lake to net and pump samples taken in the same area.

### Materials and procedures

The LOPC (Herman et al. 2004, Brooke Ocean Technology) was used to analyze both preserved net samples and in situ abundance of zooplankton. Preserved samples were analyzed using the lab circulator (Brooke Ocean Technology), which consists of a continuous flow of water through the LOPC at an approximate rate of  $0.8 \text{ L s}^{-1}$ . Preserved zooplankton samples were introduced through a chamber and recollected after passage through the LOPC on 75- $\mu\text{m}$  nitex filters. Only particles between 300–2000  $\mu\text{m}$  ESD as measured by the LOPC were included in all analyses since bubbles smaller than 300  $\mu\text{m}$  ESD were frequently present in the lab circulator, and particles larger than 2000  $\mu\text{m}$  ESD were too rare for accurate abundance calculations.

In situ abundance and biomass of zooplankton were measured by vertically hauling (by hand) the LOPC attached to a T-frame (Brooke Ocean Technology). This T-frame is designed to position the LOPC inside a 0.75-m conical plankton net for simultaneous collection by net and LOPC. However, the high concentrations of particles in our study system quickly clogged this large net, and instead, we towed the LOPC on the frame without the net, and a smaller 0.3-m 100- $\mu\text{m}$  conical net was used to take concurrent plankton net samples from the other side of the boat. Both net and LOPC tows were taken vertically, starting from 1 m above the bottom of the study lake, at a velocity of approximately  $1 \text{ m s}^{-1}$  (tow speed and consistency of speed were verified in the LOPC data output). Zooplankton samples were also collected at 2 m depth using a Waterra submersible pump (model WSP-12V-3, pump speed =  $0.25 \text{ l s}^{-1}$ )

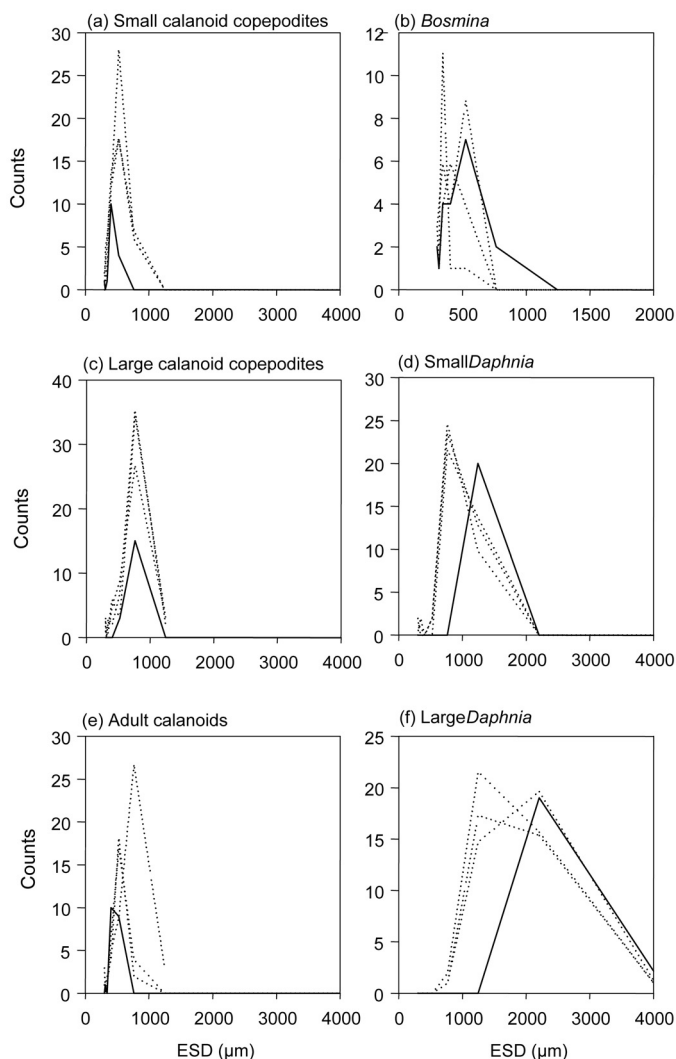
into the 0.3 m net. Ethanol-preserved net and pump samples were processed through the lab circulator in the laboratory. The precise volume of water sampled was determined using a General Oceanics Flowmeter for the nets, and the values reported in the data output file for the LOPC (based on average particle passage time through the light beam, Herman et al. 2004).

Microscope analyses and size measurements of preserved zooplankton samples were performed using an Olympus dissecting microscope and Evolution QEI Monochrome camera using ImagePro software under 20 $\times$  or 32 $\times$  magnification.

### Assessment

*Comparison of size: ESD estimates by microscope measurements and the LOPC*—Zooplankton of different species and sizes were selected from 100- $\mu\text{m}$  mesh net tow samples taken from two lakes in Quebec, Canada in 2005 (lakes Memphremagog and Petit Brompton) using a pipette under a dissecting microscope. Individuals were pooled by taxonomic group and size classes into small calanoid copepodites, large calanoid copepodites, adult calanoids (females carrying egg sacs), *Bosmina*, small-sized *Daphnia* (all from Lake Memphremagog), and large *Daphnia* (from Petit Brompton). The lengths and widths of twenty individuals from each group were measured using the imaging system. Copepodite and adult copepods were measured for total length (excluding caudal setae) and width. The length and width of the egg sacs of copepod females were also measured. For *Daphnia*, the length without spine and width as the widest point perpendicular to the length was measured, as were the length and width of *Bosmina*. ESD was calculated as the geometric mean of the measurements ( $\text{length} \times \text{width}$ )<sup>0.5</sup> (Beaulieu et al. 1999). ESD was also calculated based on measured lengths and using ratios of 3:1 and 1.6:1 length to width for copepods and cladocerans, respectively, to determine whether length measurements alone (no widths measured) could be equally used to estimate ESD. These length:width ratios are generally applied to copepods, but a similar ratio for cladoceran genera is not as common nor constant. However, length:width ratios for large *Daphnia* measured above resulted in a ratio of 1.58:1 (standard deviation = 0.32) and other studies have found a comparable ratio (1.65:1 for *D. magna* in Sosak-Swidarska et al. 1998, and 1.55:1 for *D. pulex* in Mayer and Wahl 1997). The small *Daphnia* we measured had a length:width ratio of 2.1:1, but we decided to use the ratio of 1.6:1 for simplicity and consistency with previous studies.

Approximately 30 additional animals in each group were collected, and all animals of each group were passed through the LOPC lab circulator (for a total of 30–50 individuals per group) three times. Output from the LOPC provides counts of individuals falling into 15  $\mu\text{m}$  ESD bins. The counts in these bins were grouped into larger bins (as in Beaulieu et al. 1999) with an exponential (base 2) increase in the width of size bins. The differences in average ESD of each group was compared using an ANOVA on all four estimates of ESD (one microscope



**Fig. 1.** ESD size distribution of selected groups of zooplankton using microscope measurements (solid line) and LOPC estimates (dotted lines). The three dotted lines represent the three replicate runs through the LOPC lab circulator of one sample.

and three replicate LOPC measurements) using Tukey's post-hoc test to identify where significant differences lay.

Copepod size was slightly overestimated by the LOPC while *Daphnia* size was underestimated compared to microscope measurements (Fig. 1 and Table 1). There was no significant difference between the two types of measurements for large calanoid copepodites ( $P > 0.05$ ), while the LOPC overestimated body size of smaller copepodites and adult females by about 15% (Table 1). Tukey's post-hoc test indicated that only one of three replicates of both small and adult calanoids run through the LOPC was significantly different from the microscope measurements. Within all three LOPC replicates, there were no differences. The ESDs of *Daphnia* were consistently underestimated by the LOPC by approximately 25% (Table 1) and were one size class smaller when measured using the LOPC than on the microscope (Fig. 1d,f).

Tukey's post-hoc test revealed that the microscope measurements were significantly larger than all three LOPC measurements for both groups of *Daphnia*. *Bosmina*, as measured by the LOPC, had a wide range of different size distributions (Fig. 1b), and Tukey's post-hoc test determined that only the second LOPC replicate was significantly lower than microscope estimates. It appears, however, that several of the *Bosmina* individuals fell below our 300 μm ESD lower size limit when analyzed by the LOPC (fewer than 25 of the original 50 animals were counted, Table 1), suggesting the *Bosmina*, like the *Daphnia*, were underestimated in ESD by the LOPC relative to microscope measurements. The discrepancy in numbers of animals counted by the LOPC for the other groups is comparatively small (Table 1) and is most likely accounted for by losses during sample processing.

Previous studies have also found good agreement between OPC and microscope measurements for copepods (Sprules et al. 1998, Beaulieu et al. 1999, Grant et al. 2000). Fewer studies have looked at cladoceran size with the OPC. Sprules et al. (1998) found that the OPC accurately measured ESDs for *Daphnia*, whereas Kessler and Lampert (2003) found that the OPC underestimated the size of *Daphnia hyalina*. Discrepancies have also been found for measurements of other groups of zooplankton. Sprules et al. (1998) found that *Mysis relicta* size was accurately measured by the OPC, whereas *Bythotrephes cederstroemi* size was slightly underestimated. Beaulieu et al. (1999) found that chaetognath size was underestimated by the OPC, and euphausiids were overestimated.

The differences in ESD estimates between the LOPC and microscope measurements are likely due to variations in morphology of different major groups of zooplankton. The LOPC measures size of an organism as an ESD based on the occlusion of elements in the laser beam, and is calibrated with spheres of known diameter (Herman et al. 2004). Thus, the orientation of organisms as they pass through the beam will affect the ESD measured by the LOPC. Copepods are spheroid in shape with a width and depth of approximately equal size and therefore, the orientation of the animal as it passes through the laser will affect less ESD as the animal rotates on its longitudinal axis (although rotations along other axes will affect ESD measurements). This property was also noted by Hopcroft (GLOBEC 2001) who found that OPC ESD was closely related to the prosome length of copepods. Thus, because of the consistency of copepod shape, it was not necessary to convert microscope measurements to ESD. In contrast to this, *Daphnia* and other cladocerans are laterally flattened so that the width measurement under a microscope will be much larger than the depth. Thus, as the animal rotates along the longitudinal axis (or any other axis), the ESD will be underestimated. Ultimately, the ESD calculated based on microscope counts will always be the maximum possible ESD measured with the LOPC and any differences in orientation of a cladoceran will serve to lower the ESD measurement. This underestimation, however, appears to be consistent by approximately 25% ESD,

**Table 1.** Comparison of measurements of different groups and sizes of zooplankton from Lake Memphremagog and Petit Brompton by the microscope (using length and width measurements) and the LOPC lab circulator

Group	Avg ESD Microscope ( $\mu\text{m}$ )	Avg ESD LOPC	N measured	N in sample	N counted by LOPC	ANOVA <i>P</i>
Small calanoids	380.0 (42.6)	437 (50.4)	16	48	50,46,49	0.04
Large calanoids	557.2 (53.6)	582 (80.8)	20	48	51,50,47	ns
Adult calanoids	398.5 (29.3)	452 (44.6)	20	29	29,28,24	0.027
<i>Bosmina</i>	400.5 (83.6)	358 (26.3)	20	50	25,17,18	0.023
Small <i>Daphnia</i>	964.7 (87.4)	731 (65.5)	20	50	43,37,39	<0.001
Large <i>Daphnia</i>	1644.6 (195.0)	1245 (121.4)	20	37	30,35,36	<0.001

The three numbers in the N counted by LOPC column are the number of individual counted in each of three runs of the sample through the LOPC. Numbers in parentheses represent the standard deviation of the mean. ANOVAs were performed on all four estimates of size for each group (one microscope and three LOPC lab circulator estimates). Differences as determined by Tukey's post-hoc test are discussed in the text.

and thus can be corrected for when examining systems containing predominantly cladocerans.

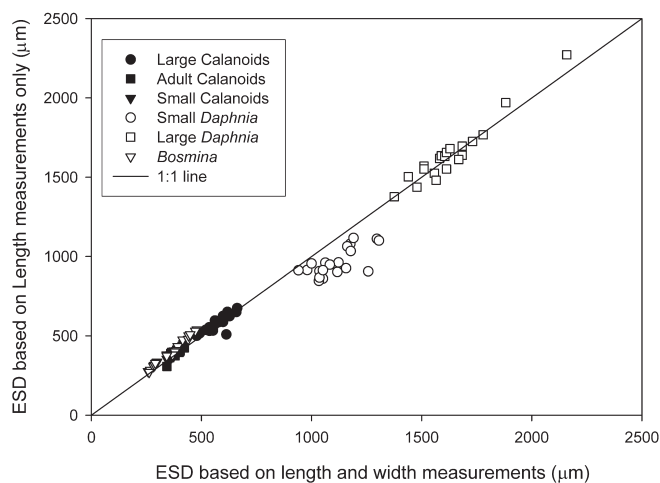
The comparison of ESD calculations based on detailed measurements of both length and width and those that use length:width ratios derived only from measures of length show very good agreement for all groups (Fig. 2). Only small-sized *Daphnia* appear to fall slightly below the 1:1 line. This result suggests that ESD estimates of small *Daphnia* would be improved using the measured ratio of 2.1:1, but it is desirable to keep the calculations as simple as possible and, therefore, no additional corrections were applied in these analyses.

*Comparison of net samples of abundance and biomass analyzed by microscope counts and the LOPC lab circulator*—Eighteen lakes in the Eastern Townships region of Quebec were sampled during the summer of 2004. In July, zooplankton samples were taken at the deep hole in each lake using a 0.5 m diameter,

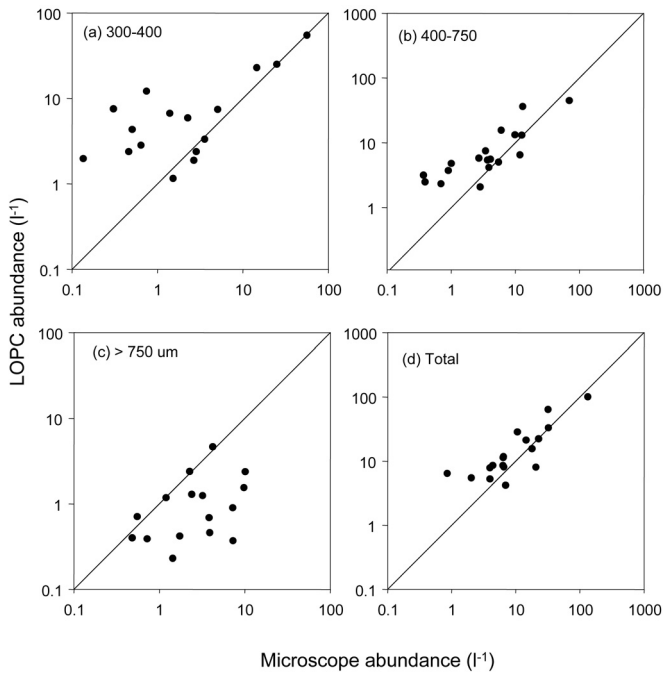
2 m long, 100- $\mu\text{m}$  mesh net from 1 m above the sediment to the surface. Cod end samples were collected and preserved in 75% ethanol. Zooplankton from sub-samples of each tow were identified to species (using Smith and Fernando 1978, Amoros 1984, De Melo and Hebert 1994, Pennak 1989, and Thorp and Covich 2001), counted for abundance, and the lengths of ten adult individuals of each species in each lake was measured using the same system and procedures described in the previous section. Juvenile copepods were also counted, but not measured for length and were not included in the abundance calculations.

For each of the 18 lakes, the ESD of adults of each species was calculated as above, using a 3:1 and 1.6:1 ratio of length:width for copepods and cladocerans. Zooplankton were grouped into four different size fractions: 300–400  $\mu\text{m}$  ESD, 400–750  $\mu\text{m}$  ESD, 750–2000  $\mu\text{m}$  ESD, and total abundance of all animals 300–2000  $\mu\text{m}$  ESD. These classes were chosen because preliminary studies suggested that the smallest fraction would include nauplii and *Bosmina* (but would underestimate the density of these zooplankton as many will be smaller than 300  $\mu\text{m}$ ), the 400–750  $\mu\text{m}$  fraction would consist mainly of copepods, and the 750–2000  $\mu\text{m}$  fraction would contain primarily large cladocerans. A sub-sample of the remaining sample was passed through the LOPC lab circulator within 1 year of collection, and abundance was calculated in the same four ESD size fractions, without correcting for known differences in ESD of cladocerans when measured using the LOPC compared to the microscope. Abundance estimates were compared using simple linear regression.

Biomass of the microscope-identified samples was calculated using published length-weight regressions of each species (Dumont et al. 1975, McCauley 1984, Culver et al. 1985, Yan and Mackie 1987) and multiplying by abundance as calculated above. To calculate biomass from the LOPC output, it is necessary to convert the ESD data into volume and convert to biomass assuming specific gravity of 1. This wet mass was converted to dry mass by multiplying by 0.2 (Peters and



**Fig. 2.** ESD calculations of six broad zooplankton groups using microscope measurements. The y-axis represents ESD as estimated using direct length measurements, and length:width ratios to calculate width, while the x-axis represents the ESD calculated from direct measurements of length and width. The 1:1 line is shown



**Fig. 3.** Abundance estimates obtained from the LOPC lab circulator and by standard microscope counts of eighteen zooplankton samples from lakes in the Eastern Townships of Quebec in (a) 300–400  $\mu\text{m}$  size fraction, results of linear regression:  $R^2 = 0.94$ ,  $P < 0.0001$ , slope = 0.93, (b) 400–750  $\mu\text{m}$ :  $R^2 = 0.7$ ,  $P < 0.0001$ , slope = 0.62, (c)  $> 750 \mu\text{m}$ , linear regression ns, (d) total:  $R^2 = 0.84$ ,  $P < 0.0001$ , slope = 0.75. The solid line represents the 1:1 line. Axes are presented in log scale to facilitate the display of the data only.

Downing 1984). ESD is often converted to a spherical volume ( $V$ ) using the equation:

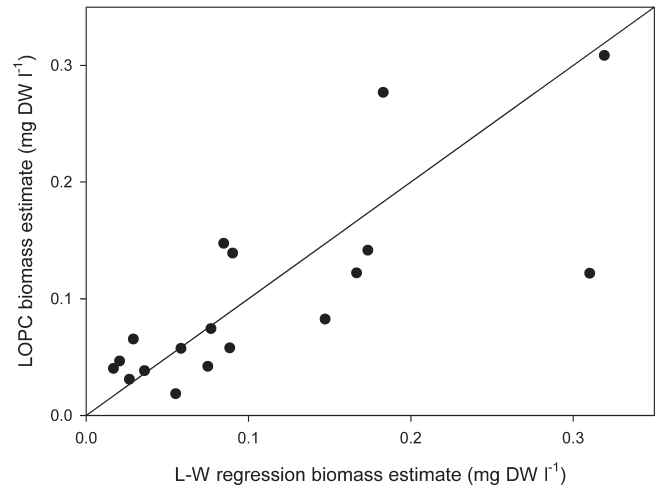
$$V = \frac{4}{3}\pi\left(\frac{ESD}{2}\right)^3 \quad (1)$$

However, as most crustacean zooplankton are ellipsoid in shape, we chose to find an appropriate length:width ratio applicable across a wide range of zooplankton shapes. As in Sprules et al. (1998), we used the equation:

$$V = \frac{\pi}{6}\left(\frac{ESD^3}{f^2}\right) \quad (2)$$

where  $f$  is the ratio of length:width. This value was iteratively changed until the lowest residual variation in the relationship of microscope-derived biomass and LOPC ESD derived biomass was found.

The relationship between the density estimates calculated using the LOPC and that calculated by traditional microscope counts was strong for all but the 750–2000  $\mu\text{m}$  size fraction (Fig. 3). The relationships for the smallest two fractions are strongly influenced by one lake (St. Georges) with very high zooplankton densities, but the significant relationships still hold when this lake is removed from the regression analyses (without St. Georges: for the 300–400  $\mu\text{m}$  size fraction,  $R^2 = 0.76$ ,  $P < 0.0001$ , slope = 0.96; for 400–750  $\mu\text{m}$ ,  $R^2 = 0.52$ , slope = 1.39,  $P < 0.0001$ ; for total  $R^2 = 0.63$ , slope = 1.19,  $P < 0.0001$ ). Sprules et al. (1998) and Beaulieu et al. (1999) similarly found good agreement



**Fig. 4.** Biomass estimates obtained from the LOPC lab circulator and species specific length-weight regressions for all zooplankton  $> 300 \mu\text{m}$  in eighteen lakes in the eastern townships of Quebec. LOPC biomass was calculated by selecting the length-width ratio of the entire community that resulted in the closest relationship with the 1:1 line (solid line).

between counts of small groups of similar organisms under the microscope and the OPC and Kessler and Lampert (2003) found the OPC accurately estimated *Daphnia hyalina* densities ranging from a few individuals to several thousand.

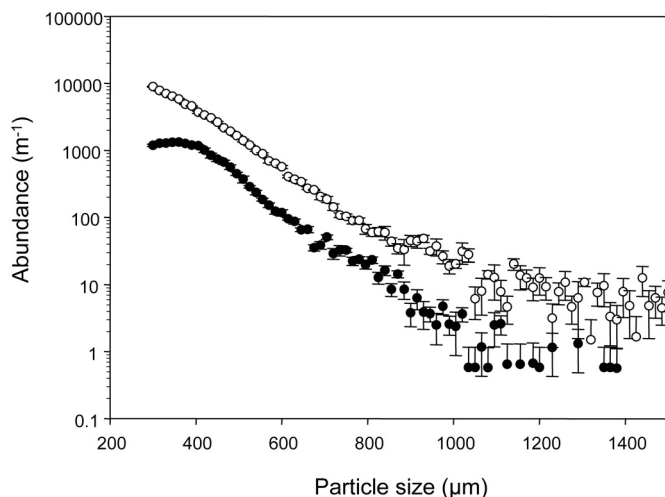
The lack of agreement in the large fraction is likely due to a combination of the underestimation of *Daphnia* ESD by the LOPC and the wide range of individual sizes. This means that it is very difficult to adequately allocate *Daphnia* to one size fraction, and that it will be particularly difficult to use the LOPC to separate different zooplankton groups in lakes with many species of varying morphologies. However, small species and total abundances are quite comparable between the two methods. Beaulieu et al. (1999) found a positive correlation between OPC biovolume and measured displacement volume of zooplankton samples, but the OPC estimate of biovolume was generally slightly lower than measured biovolume, with a slope of 0.86. The overall good agreement of total abundance of net samples between LOPC and microscope-based estimates suggests that the LOPC can be used with confidence to estimate abundance of net samples in fixed samples.

A value of  $f = 1.72$  provided the lowest residual variation in the relationship between microscope identified-derived biomass and LOPC ESD-derived biomass (Fig. 4,  $R^2 = 0.58$ ,  $P = 0.0003$ ). The one outlier in this figure is Fitch lake, the biomass of which was dominated by large *Ceriodaphnia reticulata* (responsible for 71% of biomass, with an average length = 1.75 mm). Since it was noted earlier that large cladocerans are consistently underestimated in terms of ESD by the LOPC, this likely explains the discrepancy between the two biomass estimates for this lake. When Fitch Lake is removed from the regression, the  $R^2$  increases to 0.75, and  $P < 0.0001$ . The  $f$  value changes only slightly to 1.71.

Sprules et al. (1998) originally used this method of iteratively searching for the best length:width ratio of zooplankton, which also accounted for coincident counts, and found a value for  $f$  of 1.33. Thus, our value of 1.72 does not necessarily reflect a significant change in length:width ratio of the zooplankton communities of the different study areas, but rather that coincident counts were not a factor in our analyses. Patoine et al. (2006) found good agreement between OPC, taxonomic, and seston estimates of zooplankton biomass of preserved samples and observed  $f$ -values of 2.67, 2.49, and 1.46 in eutrophic, oligotrophic, and mesotrophic lakes, respectively. They proposed that these differences in  $f$ -values were affected by the ratio of ellipsoid copepods to round cladocerans in each system, but that the relationship was not simple. They suggested that a higher cladoceran:copepod biomass ratio would result in a lower  $f$ -value and observed a smaller  $f$ -value (1.46) in the lakes with the highest cladoceran:copepod biomass ratio (1.5). We found a slightly higher  $f$ -value (1.72) at a much higher cladoceran:copepod biomass ratio (3.7 average across 18 lakes), suggesting that additional work exploring variations in  $f$ -values with varying zooplankton community composition is warranted before general trends can be reached. However, the strength of the relationship, and those in Patoine et al. (2006) suggests that biomass of lake zooplankton communities can be adequately predicted from the LOPC lab circulator analysis of net samples, once an appropriate  $f$ -value is determined for the region.

*Comparison of abundance between the LOPC in situ and preserved net samples through the lab circulator*—Vertical tows of both the LOPC and net were taken at four sites (two shallow sites at 5 m, and two deep sites at 20 m) three times a day (12:00, 19:00, 24:00) in Green Bay, a mesotrophic bay of Lake Memphremagog, Quebec (45°00'N, 72°10'W,  $z_{\text{mean}} = 20$  m,  $z_{\text{max}} = 107$  m, total phosphorus = 15  $\mu\text{g L}^{-1}$ , total nitrogen = 280  $\mu\text{g L}^{-1}$ ) on August 23 and 25, 2005. At noon on Aug 25, 2005, six replicate hauls of both the LOPC and net tows were taken at one of the deep sites to determine the coefficient of variation for both haul types. The abundances as estimated by the LOPC and net samples were compared graphically for the six replicate hauls from Aug. 25, 2005, and by performing linear regression between net tow and LOPC in situ abundance across four size fractions. Submersible pump samples were also taken on June 27 and 28, 2006, at 2 m depth and collected in a 0.3-m, 100- $\mu\text{m}$  mesh plankton net. Three replicates of 25 L were taken on 2 dates and compared to the average abundance at 2 m depth of three LOPC vertical tows.

The LOPC towed in situ estimated much higher zooplankton densities than net samples of the same water column of the one site sampled six times on Aug 25 (Fig. 5). The abundance of the smallest particles (300–400  $\mu\text{m}$  ESD) was particularly low with the net hauls compared to the LOPC tows (Fig. 5). It is not clear whether the net was capturing these particles inefficiently, or whether the LOPC was counting additional particles (i.e., detrital or algal aggregates) that may have bro-



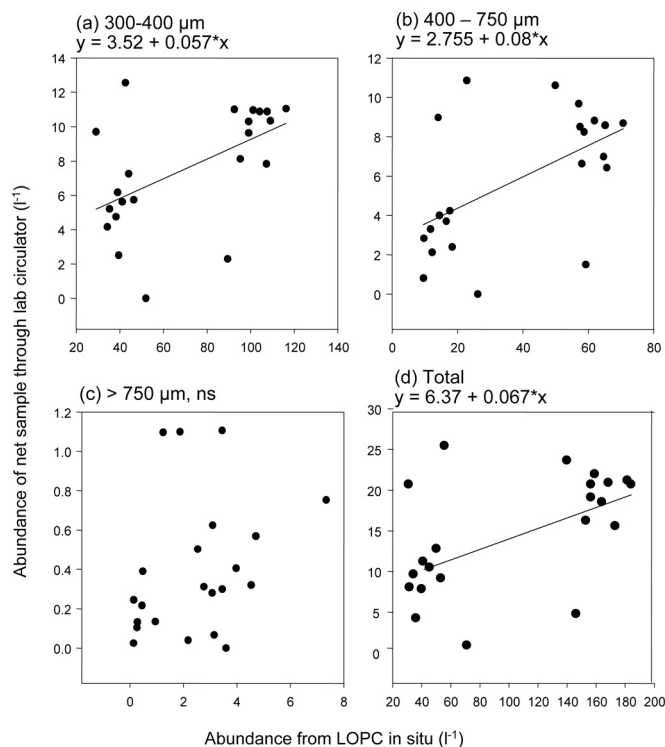
**Fig. 5.** Abundance versus size of particles in Lake Memphremagog on Aug 25, 2005, as measured by the LOPC in situ (white circles) and net tow samples preserved and run through the LOPC lab circulator (black circles). Error bars represent the standard error of six replicates.

ken up in the net. The higher abundance as calculated by the LOPC cannot be attributed to variations between tows, as evidenced by the small relative size of the error bars in Fig. 5. Furthermore, the coefficient of variation between LOPC and net tows were low, particularly for smaller size fractions of zooplankton (CV = 0.1 and 0.09 for 150–400  $\mu\text{m}$ , and 0.14 and 0.15 for 400–750  $\mu\text{m}$ , LOPC and net tows respectively).

This discrepancy between the LOPC in situ tows and the lab circulator analyses of net tows was not specific to the one site sampled six times. In general, for all size fractions, the LOPC consistently measured roughly ten times the abundance that was obtained using net samples. The regression lines noted on Fig. 6 range in slope from 0.05 and 0.075, but when forced through the origin (to fit a 1:1 expectation), slopes range from 0.11 to 0.14. The weak relationships in Fig. 6a and Fig. 6b indicated that the LOPC was counting particles that were not captured by the net. This analysis was only performed on one lake and therefore future studies will examine this relationship in more detail across different lakes.

The preserved pumped samples run through the LOPC, on the other hand, showed very good agreement with the in situ lake LOPC abundances at 2 m (Table 2). The LOPC estimated roughly double the abundance of the 300–400  $\mu\text{m}$  size fraction than the pump sample, but the 750–2000  $\mu\text{m}$  fraction abundance estimates were nearly identical between the two measurement types. Although these pump-to-LOPC comparisons are limited in number, they do suggest that the discrepancy between net and LOPC abundance estimates may lie with problems with net sampling (i.e., clogging or avoidance), rather than the LOPC counting additional phytoplankton or detrital particles.

Previous studies have also found that the OPC overestimates zooplankton abundance compared to net samples. Hopcroft (GLOBEC 2001) found that the OPC consistently counted at



**Fig. 6.** Abundance of zooplankton from four sites in Lake Memphremagog sampled three times a day for 2 days as determined by the LOPC in situ versus net samples run through the LOPC lab circulator. Note the differences in the range of axes.

least twice as many particles as the plankton net, and suggested that the OPC was including small particles (i.e., nauplii) that were not well sampled by the net. Remsen et al. (2004) found that net densities were systematically an order of magnitude less than OPC densities, which were, in turn, an order of magnitude less than Shadowed Image Particle Profiling Evaluation Recorder (SIPPER), a zooplankton-imaging system. They con-

cluded that the reason for the underestimate of OPC counts compared to SIPPER estimates was due to coincident counts, and that the discrepancy between net and OPC counts are likely due to the inability of the net to capture gelatinous zooplankton, and the OPC's inclusion of phytoplankton aggregates and marine snow. Heath (GLOBEC 2001) also found that the OPC overestimated zooplankton densities by 2.3 to 2.5 times that from a net tow due to the detection of detritus.

The presence of detritus and/or phytoplankton aggregates may be contributing to our observation of higher abundance measured by the LOPC. This may be particularly true in the 300–400 μm ESD size fractions, as the LOPC estimated disproportionately higher abundance of this size fractions compared to both the net or pump samples. The good agreement between the pump samples and the LOPC of the larger size fractions, however, suggest that inadequate sampling by the net may, in fact, explain the discrepancy between the net and LOPC abundance estimates. Although we used a conical plankton net with an open-area ratio of 4.0, Omori and Ikeda (1984) noted that an open-area ratio greater than 6.0 may be preferable in areas with high plankton densities to prevent significant avoidance and clogging of the net. Coincident counts do not appear to be responsible for our discrepancies here, since the LOPC consistently measured higher densities in situ, while coincident counts would result in an overestimation of larger particles and an underestimation of smaller particles.

One other possible source for the discrepancy of counts by the LOPC in situ and net samples is the effect of preservatives on organism size as detected by the OPC. Edvardson (GLOBEC 2001) noted that calibration should be done on live zooplankton since preservation is known to shrink animal size and affect translucence, and Beaulieu et al. (1999) and Checkley et al. (1997) found that formalin caused an increase in opacity and resulted in an increase by 3% to 25% in digital sizes of zooplankton. Black and Dodson (2003) found that there was no significant difference in shrinkage rates of *Daphnia* preserved

**Table 2.** Comparison of abundance (in particles L<sup>-1</sup>) in three size fractions as measured by the LOPC in situ and submersible pump samples at 2 m depth on (A) June 27, 2006, and (B) June 28, 2006, in Green Bay, Lake Memphremagog, Quebec.

	300-400 μm		400-750 μm		750-2000 μm	
	LOPC	Pump	LOPC	Pump	LOPC	Pump
<b>A</b>						
1	89.2	45.4	40.6	45.1	8.4	6.5
2	106.3	46.2	59.1	45.6	5.5	8.5
3	153.0	38.3	65.6	42.3	7.7	6.3
Average	116.2	43.3	55.1	44.3	7.2	7.1
<b>B</b>						
1	76.9	29.4	67.2	30.4	5.5	6.2
2	116.5	38.9	60.3	37.4	3.8	4.4
3	106.9	39.6	49.7	41.9	4.4	2.5
Average	100.1	35.9	59.1	36.6	4.6	4.4

in ethanol over formalin and found that ethanol often caused the *Daphnia* to be “tinted yellow” after 18 months in ethanol preservation. We compared size spectra of three zooplankton samples before and 24 h after preservation in ethanol and found that while total counts of particles didn’t change, the smallest size fraction (300–400  $\mu\text{m}$  ESD) was approximately 10% lower in abundance after preservation (data not shown). Thus, preservation may increase the ESD as detected by the LOPC. This could explain part of the discrepancy between the pumped and LOPC in situ counts, but is not sufficient to explain the two times difference in the 300–400  $\mu\text{m}$  size fraction, nor the differences between the vertical net hauls and the LOPC in situ. Future studies will examine the effects of long-term preservation on size and abundance estimates.

### Discussion

Overall, the LOPC gave very good estimates of fixed net sample zooplankton abundance across lakes with very different species assemblages and densities up to 140 individuals per liter. Thus, the presence of other particles in preserved net samples such as detritus and large phytoplankton likely contributed to the variation in the comparisons but were not drastic enough to confound the comparisons. Patoine et al. (2006) similarly found that phytoplankton were not affecting comparison of zooplankton biomass estimates and suggested that phytoplankton is not well detected by the OPC. The good agreement between pump samples and the LOPC in situ is promising for future studies in lakes, but the discrepancy with net samples suggests that the use of the LOPC in situ should be used cautiously.

The orientation of particles through the LOPC still appears to be a concern for estimates of zooplankton size. While the LOPC estimated copepod size was found to correspond with microscope values, cladoceran size was consistently underestimated. Overall, with one exception, this underestimate did not drastically affect whole community abundance or biomass estimates. It has been demonstrated that preservation may also affect the size of zooplankton relative to live zooplankton in situ, but as with the underestimation of cladoceran ESD by the LOPC, this may not significantly affect community abundance and biomass estimates. Future studies will examine in more detail to what degree long-term preservation in ethanol ultimately affects zooplankton abundance and biomass estimates.

The LOPC appears to have overcome the concerns of incorrect volume estimation and coincident counts that plagued its predecessor, the OPC (Sprules et al. 1998, Woodd-Walker et al. 2000). Sprules et al. (1998) found that coincident counts influenced OPC counts at zooplankton abundance of 10–30  $\text{L}^{-1}$ . We saw no evidence of coincident counts in our in situ analyses in Lake Memphremagog, where zooplankton abundance reached 25  $\text{L}^{-1}$  (net sample estimate, LOPC estimate reached 180  $\text{L}^{-1}$ ). This reduction in coincident counts is encouraging and suggests that the LOPC is accurately counting all particles in the water.

### Comments and recommendations

The results here suggest that the LOPC and its lab circulator can currently be used with great confidence to analyze preserved net samples of zooplankton. While the LOPC did tend to underestimate cladoceran size compared with microscope measurements, it did so consistently, and did not appear to significantly alter total abundance or biomass estimates in most cases. However, it is necessary to know at least basic information about zooplankton composition in study lakes since it was found that the LOPC underestimated the biomass of one lake dominated by very large cladocerans.

There is also evidence that the LOPC contains great improvements (most significantly of which is the reduction in coincident counts), which will allow for direct measurements of zooplankton size and density in situ. Future studies using the LOPC will need to consider the presence of larger detrital or algal aggregates, which may confound estimates of zooplankton abundance, particularly in size fractions < 400  $\mu\text{m}$  ESD. The comparison of additional net and pump samples with the LOPC towed in situ across lakes with a range of abundances of zooplankton and detrital aggregates and phytoplankton colonies will allow for further comparisons between sampling strategies to increase our confidence in the data from the LOPC. In addition, more information regarding the effect of long-term preservation must be examined in order to evaluate this as another possible source of error. Finally, we have not used the LOPC to its full potential in this study here, as we only examined particles larger than 300  $\mu\text{m}$  ESD. The LOPC has a lower detection limit of 100  $\mu\text{m}$ , which allows as well for sampling of copepod nauplii and large rotifers, crucial components of the zooplankton community. The evaluation of the LOPC to accurately estimate abundance and biomass of these small particles is the final step necessary in order to make the LOPC a routine and reliable instrument for sampling of zooplankton communities in lakes.

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