

NOAA Technical Memorandum GLERL-143

**SELECTING OPTICAL PLANKTON COUNTER SIZE BINS TO
OPTIMIZE ZOOPLANKTON INFORMATION IN GREAT LAKES
STUDIES**

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August 2008



UNITED STATES
DEPARTMENT OF COMMERCE

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NATIONAL OCEANIC AND
ATMOSPHERIC ADMINISTRATION

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Selecting Optical Plankton Counter Size Bins to Optimize Zooplankton Information in Great Lakes Studies

James R. Liebig and Henry A. Vanderploeg

Abstract. Equivalent spherical diameter (ESD) size bins for the optical plankton counter (OPC) were determined so as to optimize the OPC for providing abundance of Great Lakes zooplankton species. By determining OPC ESDs for animals of known lengths (L) pumped through laboratory OPC, we derived the ESD/L ratio for a variety of species. Based on the size spectra of individual species lengths determined from net tows and application of previously determined ESD/L ratios, divisions for OPC size bins were selected so as to separate species or groups of species. Since there are differences in zooplankton composition and size within species between Lakes Michigan and Erie, the OPC size bins designated were not the same in the two lakes. This report describes the process used to determine OPC bin sizes and presents the results of an example from Lake Michigan and one from Lake Erie. In addition, ESDs calculated from zooplankton lengths may provide a size metric more useful than length because an ESD more closely resembles visual target strength than length.

1. INTRODUCTION

Developed for marine systems more than 20 years ago (Vanderploeg and Roman 2006), the optical plankton counter (OPC) has been used in the Great Lakes for more than 10 years (Stockwell and Sprules 1995) to provide information on spatial distribution of zooplankton biomass and zooplankton community size structure, but has not revealed much about zooplankton species composition (e.g. Sprules et al. 1998, Zhou et al. 2001, Yurista et al. 2006). Individual species usually cannot be distinguished in OPC size-frequency distributions because there is often a significant size overlap among species. The OPC can be used as a tool for estimating the spatial distribution of specific taxa if appropriate size bins are selected to separate those taxa. By determining OPC ESDs for animals of known lengths (L) pumped through laboratory OPC, we determined the ESD/L ratio for a variety of species. There is no single universal ESD/L ratio because of the many different shapes of zooplankton in the Great Lakes. Based on the size spectra of individual species OPC ESDs, calculated from experimentally determined ESD/L ratios or estimated from measured dimensions, divisions for OPC ESD size bins were selected so as to separate species or groups of species. These binning techniques can be used in various studies to help analyze data collected by the OPC. Selective binning of OPC data from a particular lake allows us to produce depth profiles of the major zooplankton species, and correlate various taxa with temperature and other environmental data. Gathering the OPC data over long transects and over 24 hour periods can reveal insights about spatial distribution and diel vertical migration. Results from specific studies will be presented in other papers. This report focuses on methods used to determine bin sizes and presents an example from Lake Michigan and Lake Erie.

2. METHODS

Field Procedures

To illustrate the process and application of bin sizing, data from 153- μm and 64- μm mesh zooplankton net samples from Lake Michigan and Lake Erie respectively and OPC tows in both lakes were utilized. Lake Michigan data was collected along an inshore/offshore transect (10-m to 60-m deep) west of Muskegon, Michigan in August 2004. Lake Erie data were from the central basin at offshore sites up to 24-m deep north of Cleveland, Ohio in August 2005.

Instruments

The OPC (Model 2T, Focal Technologies, Inc.) used in this study is mounted on a V-fin (Endeco/YSI) as part of a plankton survey system (PSS) (Ruberg et al. 2001), which also includes a mechanical flow meter (General Oceanics), an Aquatracka III fluorometer (Chelsea Technology Group), an OS200 CTD (Ocean Sensors), and a photosynthetically active radiation (PAR) sensor. To obtain vertical as well as horizontal spatial structure, the PSS was continuously lowered and raised at $\sim 0.25 \text{ m s}^{-1}$ in a sinusoidal path between 1-2 m beneath the lake surface and 1-2 m above the bottom with the OPC logging data every 0.5 s as the boat moved at $\sim 2.5 \text{ m s}^{-1}$ along the transect.

Zooplankton size and abundance data were collected by the OPC component of the PSS. Herman (1992) has described the OPC-1T that operates in the same manner as our OPC-2T. The OPC-2T is different in that the sampling tunnel, instead of being 25-cm wide, is only 10-cm wide to reduce coincidence (Focal 1996). Each particle between 0.25 and 14 mm equivalent spherical diameter (ESD) going through the OPC generates a signal proportional to the area of shadow cast as it passes a light beam and the OPC software converts the digital output to ESD. The generated ESD is actually the same as equivalent circular diameter (ECD) based on the area of a zooplankton's silhouette and, for non-spherical particles such as zooplankton, is not necessarily equal to the zooplankton's true ESD (Sprules et al. 1998, Beaulieu et al. 1999). Depending on the orientation of zooplankton as they pass the detector, ESD, and thus biomass (wet, based on volume assuming a density of 1.0 g cm^{-3}), calculated by the standard OPC software may be underestimated or overestimated (Liebig et al. 2006). However, zooplankton appear to go through the OPC in random orientation, which produces an approximation of their true ESD and biomass on average (Wieland et al. 1997, Sprules et al. 1998, Liebig et al. 2006).

Estimation of Zooplankton ESD/L Ratios

To determine ESD/L ratios of the different species, the laboratory OPC (Model 1L, Focal Technologies Inc.) was used to generate ESDs. The basic operating principle of the laboratory unit is the same as for the field unit (Model 2T), but the sensing zone is 2-cm wide (1.6 ml) instead of the 10-cm wide (8 ml) for the OPC-2T (Focal 1996). To perform tests in the laboratory, we modified the design of a circulator system suggested by Focal Technologies (MacKay 1996) and circulated through the OPC animals from a single taxonomic group, individually and in aliquots of 100-200 animals per test (Liebig et al. 2006). Zooplankters were added gradually to keep the concentration low so that coincidence would not be a factor, and they were recovered using a 100- μm sieve after passing once through the OPC. They were counted and measured under a microscope with a video camera using Image-Pro Plus (Media Cybernetics) imaging software. By measuring the length of each zooplankton in the test group and comparing the lengths to their OPC ESD spectrum, ESD/L ratios were obtained for *Daphnia* sp., *Bythotrephes longimanus*, *Limnocalanus macrurus*, *Diaptomus* spp., and mixed cyclopoids. Since it was not always possible to match a particular individual's length with its individual OPC signature, a simple ratio of the means of ESDs and lengths for each taxon was calculated (Table 1). For other taxa not included in lab OPC tests, an OPC ESD/L ratio was approximated by calculating an individual's ESD from its volume since calculated ESDs are nearly the same as OPC measured ESDs (Liebig et al. 2006), and the assumption is that animals of a similar size and shape have the same ESD/L ratio. In these cases, the volume (V) and ESD of an individual was calculated using the following equations assuming an ellipsoid shape using its measured length, width (either measured or calculated using length/width ratios determined from published taxon-specific lengths and widths (Malley et al. 1989)), and depth (calculated using length/depth ratios determined from published taxon-specific lengths and depths (Malley et al. 1989) or, if not available, assumed to be the same as width):

$$(1) \quad V = (\pi/6) * L * W * D$$

where V = volume, L = length, W = width, D = depth, and

$$(2) \quad ESD = (6V/\pi)^{1/3},$$

where ESD = equivalent spherical diameter, and V = volume.

Bin Size Selection

To determine what zooplankton taxa were detected by the OPC in a particular lake or study region, the species composition was identified from net collections. The objective was to separate major taxa into different OPC size bins. Vertical plankton net tows of the full water column were used to determine zooplankton species composition in the lake or region of interest. Using the methods described in the section above, length measurements of at least 25 random individuals from each of the most abundant taxonomic groups were used to calculate ESDs for size frequency distributions. If widths and depths could not be obtained, the volume (and associated ESD) was estimated from calculated mass using published taxon-specific length-mass regressions (Culver 1985, Malley et al. 1989). There was usually much size overlap among all zooplankton species from the community, especially among smaller ones. To make it easier to determine boundaries for size bins, instead of using all of the species, only selected

Table 1. Relationship of optical plankton counter (OPC) equivalent spherical diameter (ESD) to microscope measured length (L) ratios for major taxa of different length/width (L/W) ratios. The L/W ratios are approximations for a taxonomic group and meant to depict the general shape of an animal, with W representing, in this case, a rough mean of width and depth.

Taxon	L/W ratio	ESD/L	Length measurement criteria
<i>Bosminidae</i>	1.5:1	0.76 ^a	Anterior margin of head to posterior margin of valves
<i>Bythotrephes</i>	2.0:1	0.603 ^b	Distal margin of head to base of posterior spine
<i>Cercopagis</i>	2.0:1	0.603 ^c	Distal margin of head to base of posterior spine
Calanoid copepods	3.8:1	0.404 ^b	Anterior margin of head to posterior margin of caudal rami
Cyclopoid copepods	3.5:1	0.423 ^b	Anterior margin of head to posterior margin of caudal rami
<i>Daphnia</i> spp.	2.0:1	0.566 ^b	Anterior margin of head to base of posterior spine
<i>Diaphanosoma</i>	2.5:1	0.54 ^a	Anterior margin of head to posterior margin of valves
<i>Leptodora</i>	6.0:1	0.30 ^a	Anterior margin of head to fork in postabdomen
Lg. <i>Chydoridae</i>	1.5:1	0.76 ^a	Distal margin of head to posterior margin of valves
Sm. <i>Chydoridae</i>	1.3:1	0.84 ^a	Distal margin of head to posterior margin of valves

^a estimate, based on ESD calculated from ellipsoid volume determined by length and L/W ratio.

^b OPC-determined ESD and microscope-measured length from lab OPC test.

^c used same ratio as *Bythotrephes*.

major taxa were used. Furthermore, the major taxa were the ones in which we were interested in tracking, and they contained most of the zooplankton biomass. Based on the calculated ESD size spectra of individual species or groups of species, divisions for OPC size bins were selected at points where there was minimal overlap of major taxa. These size bins were applied to OPC data for that lake or region.

Applying Species Information to OPC Depth Profile Data

At specific sites, the objective was to identify species or taxonomic groups at different depth strata using the OPC. Knowledge of the species present, relative abundance, size, and preferred habitat are required to optimize zooplankton information obtained from OPC data to construct a species depth profile at a specific site and time. From a full water column net tow sample collected at the same time (within an hour or two) and station as the OPC data, ESDs for each species were calculated. ESD size biomass distributions were produced for dominant taxa (i.e. taxa with the highest relative biomass). Using that information, we determined which species or taxonomic group best represented each OPC size bin at any particular time and depth. There are usually more species at the small end of the size spectrum, and the bin containing the smallest sizes often could not be identified as any particular one or two species or taxonomic groups.

3. RESULTS AND DISCUSSION

Lake Michigan Example

Overall, in zooplankton net samples collected along the inshore/offshore transect in Lake Michigan in August 2004, there were four main taxonomic groups with high relative biomass that had reasonable size separation: *Bythotrephes* sp., *Daphnia* spp., *Bosmina* sp., and *Leptodiptomus sicilis*. Based on the calculated ESD size distribution of these groups (Figure 1), we selected bin divisions to separate the taxa for associated OPC data. The bin sizes were 0.25 - 0.50 mm (*Bosmina*), 0.50–0.75 mm (*Leptodiptomus* and small *Daphnia*), 0.75–1.50 mm (large *Daphnia*), and 1.50–3.50 mm (*Bythotrephes*) for bins 1, 2, 3, and 4 respectively (Table 2). Since there is a complete overlap in their sizes, the OPC cannot be used to distinguish *Leptodiptomus sicilis* from small *Daphnia*. Note also that much of the *Bosmina* biomass fell below 0.25 mm, the minimum size threshold of detection for the OPC.

To examine a more specific place and date, in this case station M60 (offshore, 60 m depth) in Lake Michigan on August 3, 2004, ESDs for the taxa found in a full water column plankton net samples were determined. Biomass was calculated from measured lengths for abundant species and for less abundant species, previously determined default biomass values (unpublished data) were used. To get a quick overview of the site, mean ESDs were derived from mean individual biomass since that had already been generated from a prior analysis. Figure 2 shows mean ESD of the taxa and in what bins they would likely be detected based on the previously determined bin divisions. The variance around the mean ESD of each taxon could not be determined because mean ESD was calculated from mean biomass, not from each individual. However, it is obvious that some species would fall into two size bins and many taxa overlap, especially when you consider that there is variation in size around the mean, as evidenced in Figure 1 showing the size spectra of dominant species from composite samples. The point is that more information is needed in order to give a size bin any kind of taxonomic identity. Depending on the total biomass present for each species, bins 3 and 4 and possibly 2 may each represent a single taxon, however bin 1 contains many taxa which overlap in size and is less likely to have a clear dominant in that range. Table 2 shows the bins with their size ranges and the taxa that are likely to occur in those bins for Lake Michigan.

Total biomass for each species (Figure 3) was examined to determine the dominant taxa (i.e. those with highest biomass). The size biomass distribution of *Bythotrephes longimanus*, *Daphnia mendotae*, *Limnocalanus macrurus*, *Leptodiptomus sicilis*, and *Diptomus* copepodites relative to total biomass

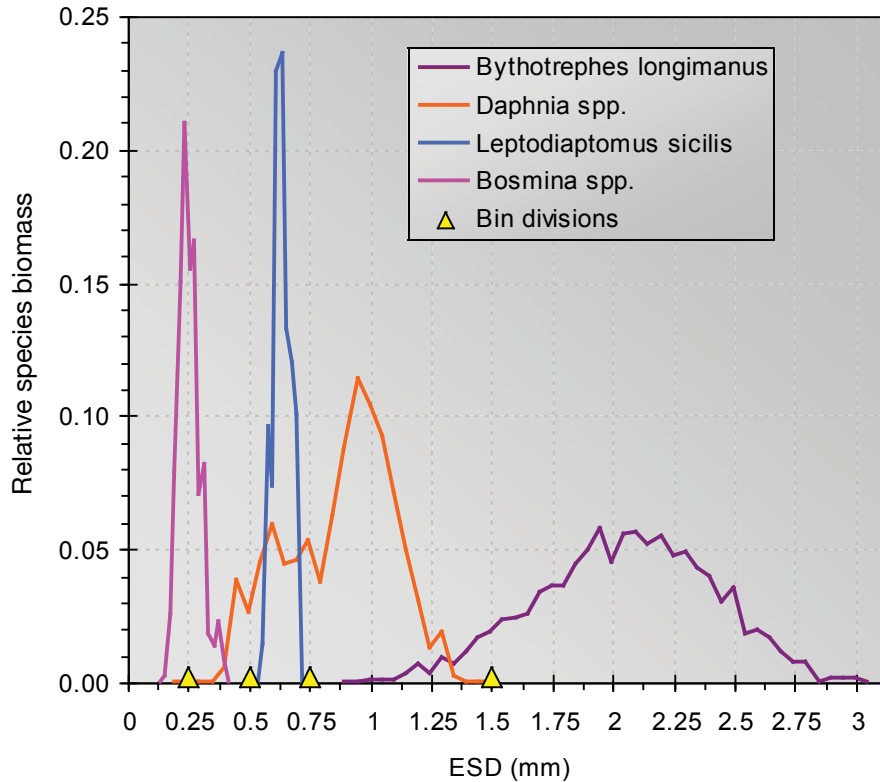


Figure 1. Size spectra (relative biomass at each ESD within each taxon) of *Bosmina* spp. (n=351), *Daphnia* spp. (n=952), *Leptodiaptomus sicilis* (n=44), and *Bythotrephes longimanus* (n=1396) from a composite of net samples in Lake Michigan. The yellow triangles are bin divisions and were placed so as to separate species.

Table 2. OPC size bins for Lake Michigan and taxa likely to fall within those bins.

	Range (mm)	Nearshore zooplankton in range	Offshore zooplankton in range
Bin 1	0.25 - 0.50	<i>nauplii</i> , <i>Bosmina</i> , copepodites, small copepods, small chydorids	<i>nauplii</i> , copepodites, small copepods
Bin 2	0.50 - 0.75	<i>Leptodiaptomus sicilis</i> , small <i>Daphnia</i> spp., <i>Cercopagis</i>	<i>Leptodiaptomus sicilis</i> , small <i>Daphnia</i> spp., <i>Leptodora</i> *
Bin 3	0.75 - 1.50	large <i>Daphnia mendotae</i> , <i>Cercopagis</i>	large <i>Daphnia mendotae</i> , <i>Limnocalanus</i> , <i>Leptodora</i> *
Bin 4	1.50 - 3.50	<i>Bythotrephes longimanus</i>	<i>Bythotrephes</i> , <i>Mysis</i>

*Note: *Leptodora* were not included in laboratory OPC tests, but adult *Leptodora* should show up mainly in bin 3 based on their size. However, due to their clarity, they may actually be detected mainly in the bin 2 size range.

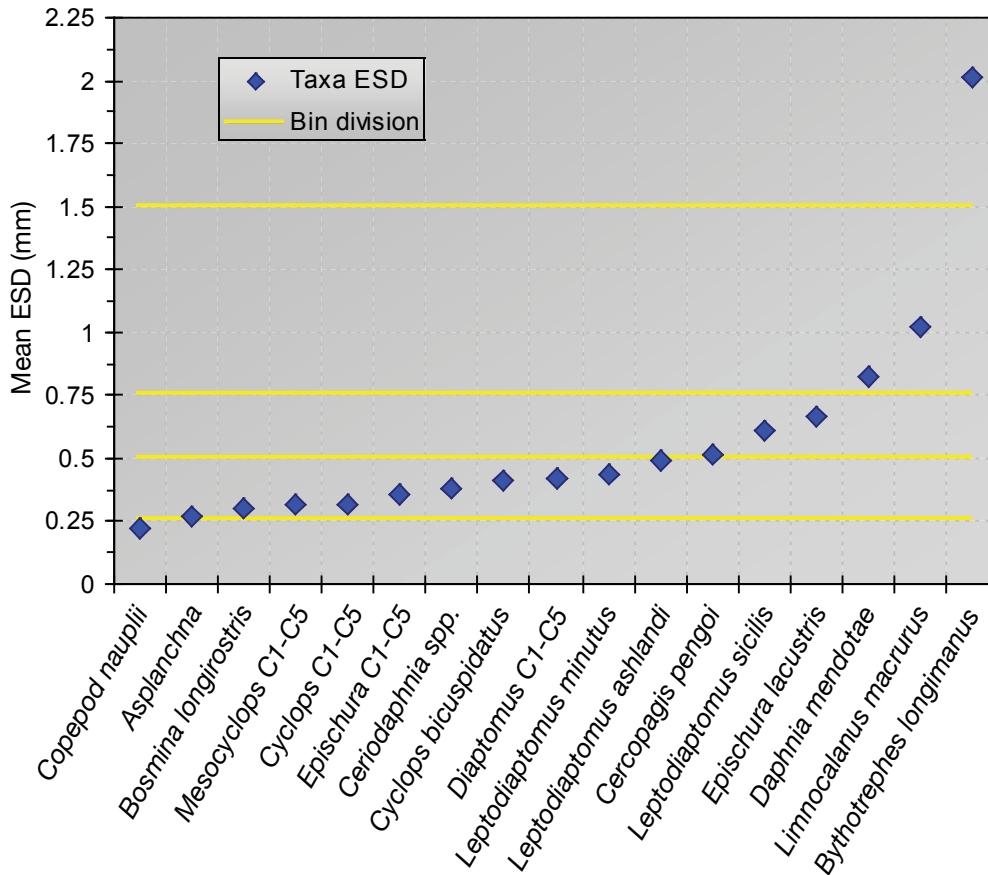


Figure 2. Mean ESD (mm) estimated from mean individual biomass of taxa found in a plankton net sample from station M60 in Lake Michigan on August 3, 2004. The yellow lines are the previously designated OPC bin divisions to show approximately how the taxa would be binned.

of the plankton net sample was plotted (Figure 4) to determine how they were distributed in the existing OPC bins. The *Leptodiaptomus ashlandi* size distribution was not plotted due to lack of length measurements. The remaining other species (black squares in Figure 3) were insignificant in terms of biomass. Basically, calanoid copepods and small *Daphnia* spp. dominate bins 1 and 2 (0.25-0.50 mm and 0.50-0.75 mm), *Limnocalanus* and large *Daphnia* comprise bin 3 (0.75-1.5 mm), and bin 4 is *Bythotrephes* (1.5-3.5 mm). The boundaries could have been adjusted at this point to better fit the size spectra of certain taxa, but it is nice to have the same size bins for a lake or large region for comparison among sites and also to lessen the amount of OPC data processing. The shape of the biomass distribution for the major taxa from the net samples is approximated by the shape of the OPC biomass spectrum (Figure 5), so it appears that the designated ‘major taxa’ characterized the OPC spectrum fairly well. The OPC spectrum is broader than the net sample spectrum because the random orientation of zooplankton in the OPC sensing zone causes ESDs to be both underestimated and overestimated (Liebig et al. 2006). Note also that some biomass from very small and/or less abundant zooplankton are not included in the net sample spectrum.

The OPC zooplankton depth profile for a particular station can be plotted showing the biomass distribution for each bin. In the plot of bins 3 and 4 (Figure 6), we figured that *Bythotrephes* were in

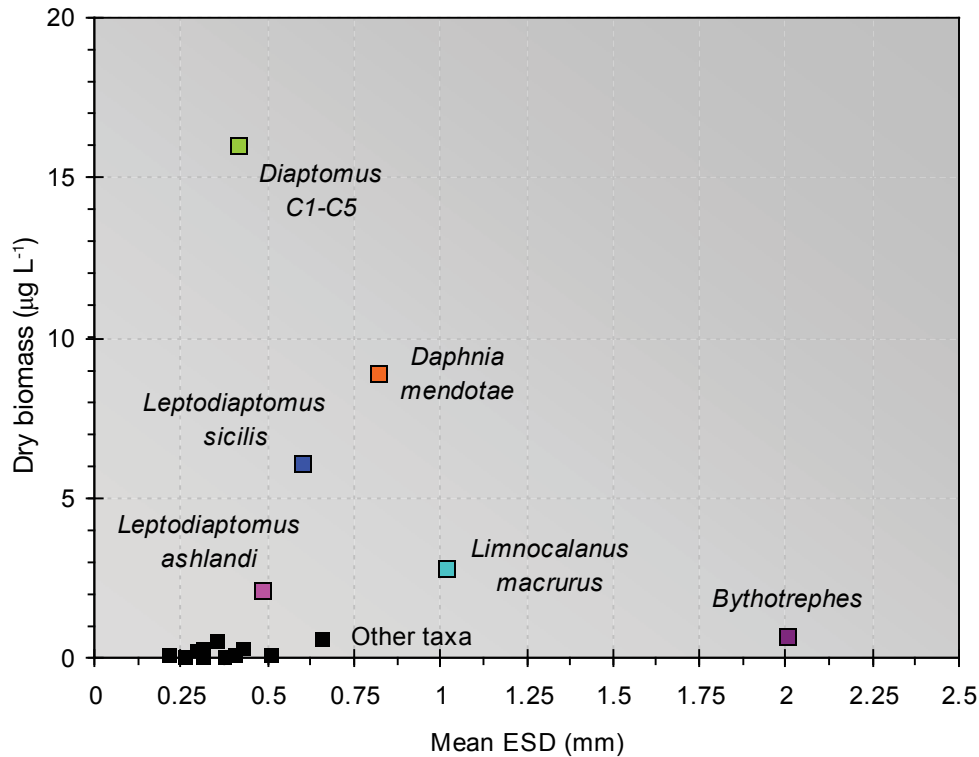


Figure 3. Total dry biomass ($\mu\text{g L}^{-1}$) of the major species (colored squares) and the remaining other taxa (black squares) species found in plankton net samples from station M60 in Lake Michigan on August 3, 2004.

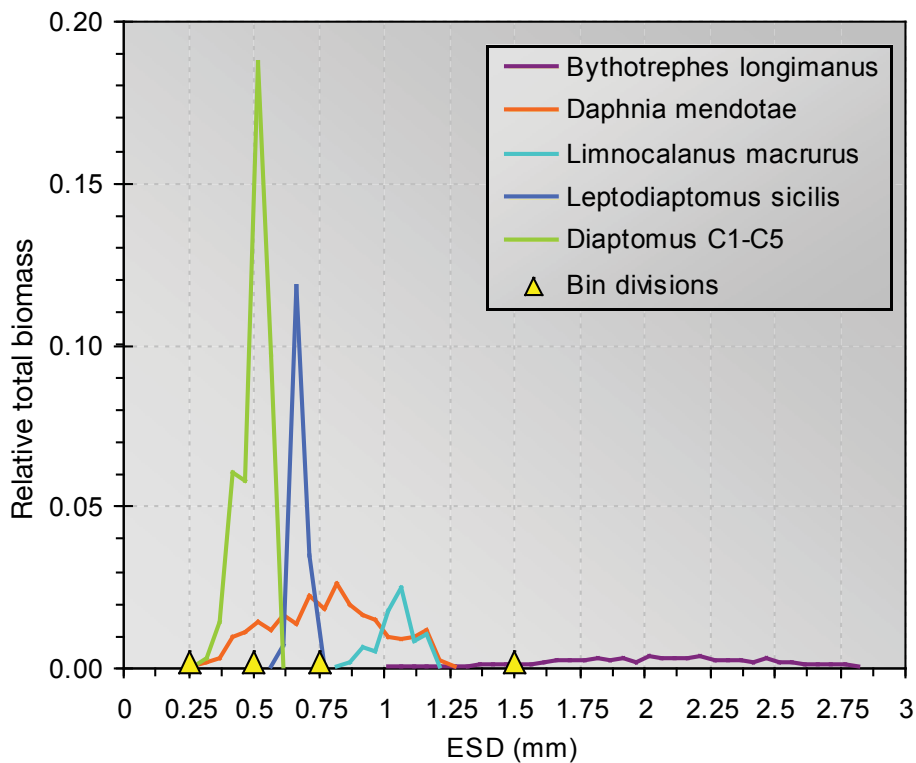


Figure 4. Size biomass distribution of *Bythotrephes longimanus*, *Daphnia mendotae*, *Limnocalanus macrurus*, *Leptodiaptomus sicilis*, and *Diaptomus* copepodites relative to total biomass of a plankton net sample from station M60 in Lake Michigan on August 3, 2004. Note that these are the dominant species, which comprise most of the biomass at this station. The yellow triangles show the previously determined bin divisions.

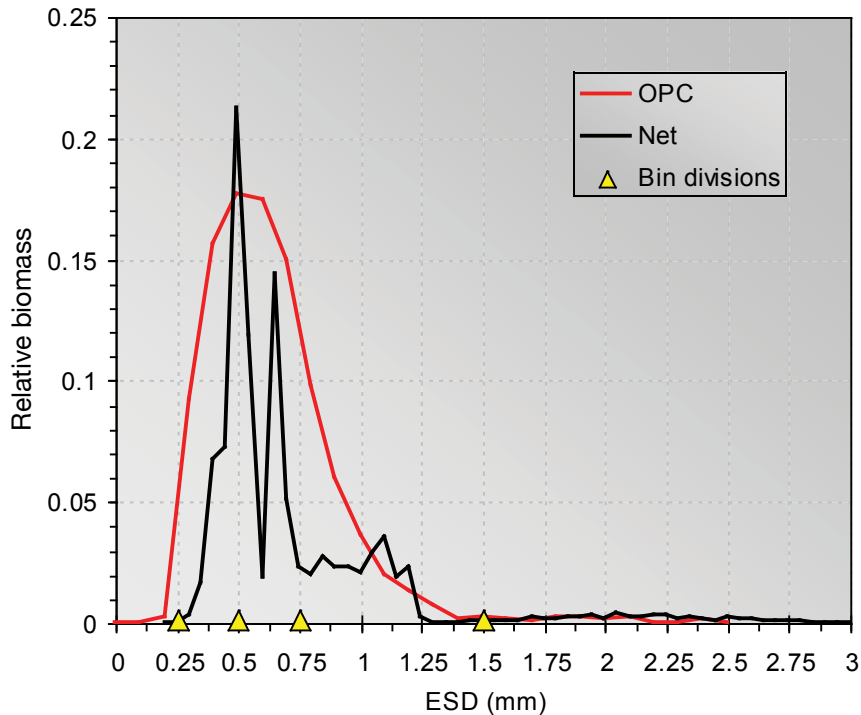


Figure 5. Comparison of OPC biomass spectrum (red) from M60 on Aug. 3, 2004 with total relative biomass from a corresponding plankton net sample (black, i.e. sum of individual species curves from Figure 4). The yellow triangles show the previously determined bin divisions.

groups throughout the epilimnion and metalimnion, and there is a concentration of large *Daphnia* at the bottom of the metalimnion with some perhaps into the upper hypolimnion. *Limnocalanus* were throughout the hypolimnion. Since *Limnocalanus* and large *Daphnia* fell into the same OPC size bin, their locations were inferred based on previous knowledge of their habits. This was confirmed with separate net samples from the epilimnion, metalimnion, and hypolimnion. However, OPC data are much finer depth scale than data obtained from net samples. Biomass in bins 1 and 2 (not shown) is fairly uniform throughout the water column and, given the overlap in sizes of several species, no species information could be inferred.

Lake Erie Example

The zooplankton community of Lake Erie was more diverse than that of Lake Michigan, and zooplankton as a whole were smaller than those of Lake Michigan (Figure 7). The size difference was due to the presence of small species and because mean sizes of individuals of a given species present in both systems were smaller (e.g. *D. mendotae* and *B. longimanus*) in Lake Erie. Thus, different OPC size bins were selected for Lake Erie (Figure 7, Table 3).

The OPC zooplankton depth profile for a station in Lake Erie shows the biomass distribution for bins 3 and 4 (Figure 8). Net samples contained no *Limnocalanus* or *Leptodora*, so we concluded that *Bythotrephes* were in bin 3 and occurred mainly in the metalimnion. Bin 2 biomass was mostly *Daphnia* and occurred in most of the water column with biomass peaks at the middle of the epilimnion and at the bottom half of the metalimnion into the upper hypolimnion. Biomass in bin 1 (not shown) is fairly uniform throughout the water column and, given the overlap in size of several species, no species information could be inferred.

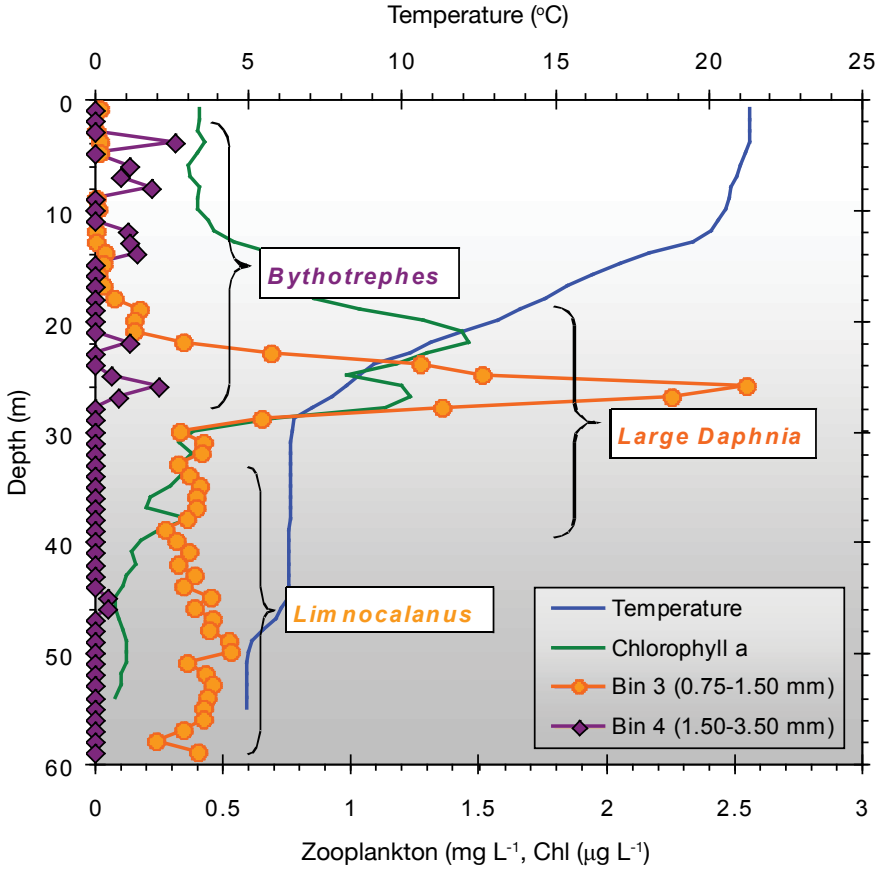


Figure 6. PSS depth profile (mean data for 1 meter intervals) of temperature ($^{\circ}\text{C}$), chlorophyll ($\mu\text{g L}^{-1}$), and zooplankton wet biomass (mg L^{-1}) in bins 3 and 4 from OPC survey at ~20:00 EDT on August 3, 2004 at station M60 in Lake Michigan.

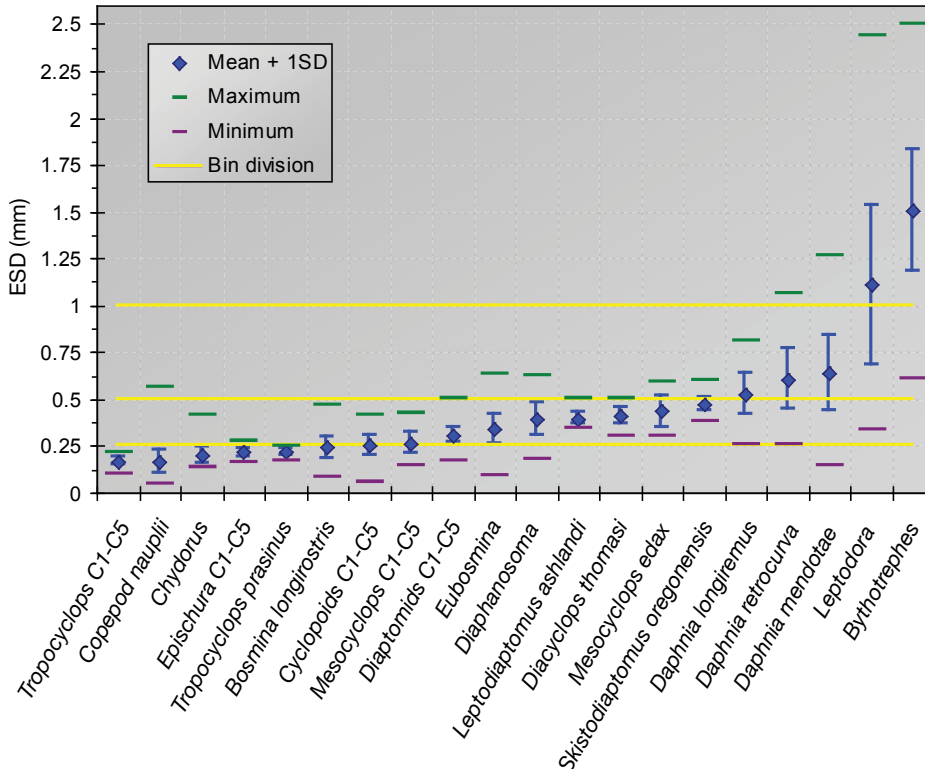


Figure 7. Maximum, minimum, and mean (± 1 SD) ESD (mm) of taxa found in plankton net samples from several stations in Lake Erie, June through September 2005. ESDs of individuals were determined from ESD/L ratios. The yellow lines are the designated OPC bin divisions.

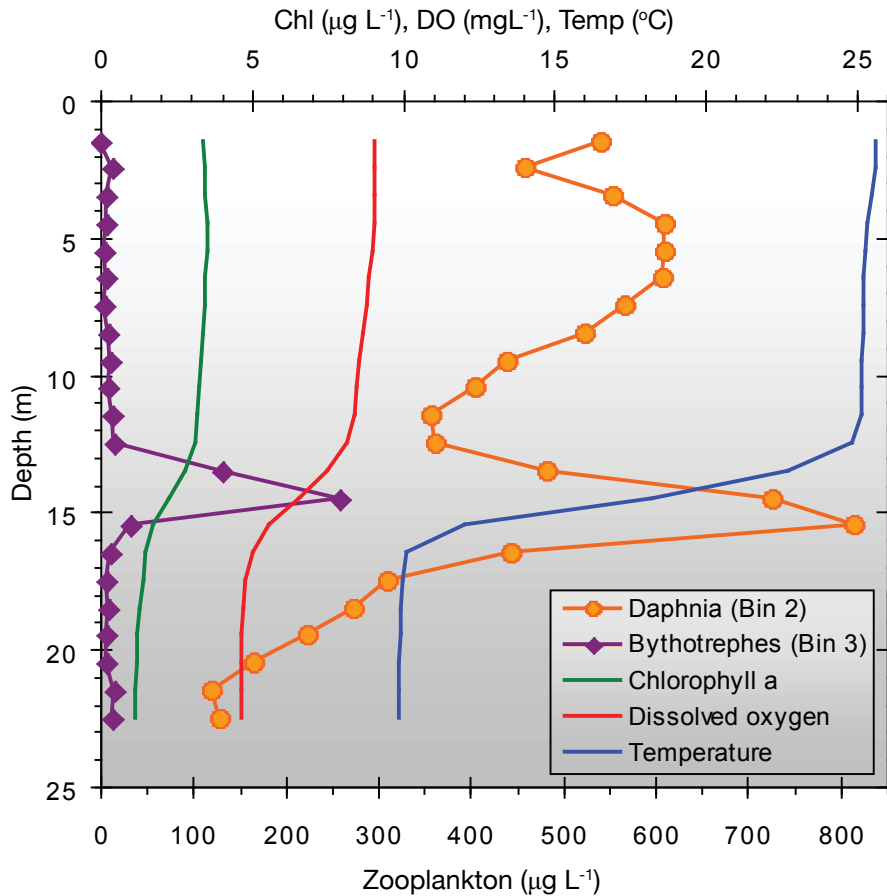


Figure 8. PSS depth profile (mean data for 1 meter intervals) of zooplankton wet biomass ($\mu\text{g L}^{-1}$), chlorophyll ($\mu\text{g L}^{-1}$), dissolved oxygen ($\mu\text{g L}^{-1}$), and temperature ($^{\circ}\text{C}$) in bins 3 (0.50 - 1.00 mm) and 4 (1.00 - 4.00 mm) from OPC survey at ~21:00 EDT on August 16, 2005 at site B in the deepest part of the central basin of Lake Erie.

Table 3. Derived OPC size bins for Lake Erie and taxa likely to fall within those bins.

	Range (mm)	Zooplankton taxa in range
Bin 1	0.25 - 0.50	<i>nauplii, Bosmina, copepodites, copepods, Chydorus</i>
Bin 2	0.50 - 1.00	large <i>Daphnia, Limnocalanus, Leptodora*</i>
Bin 3	1.00 - 4.00	<i>Bythotrephes, Leptodora*</i>

*Note: *Leptodora* were not included in laboratory OPC tests, but adult *Leptodora* should show up mainly in bin 3 based on their size. However, due to their clarity, they may actually be detected mainly in the bin 2 size range.

4. CONCLUSIONS

Plankton net samples are very important in determining the zooplankton species that are represented by a particular OPC size bin. Generally, without additional information, individual species cannot be distinguished from an OPC size spectrum because there is too much overlap in the size spectra of each species, and the OPC tends to smear the data. However, proper size binning can help determine where particular taxa are within the spectrum. Using species information from net samples in conjunction with OPC tows can help determine spatial and temporal distribution of specific or groups of taxa. In our studies, dominant species could be tracked by the OPC because they were known (based on net samples) to be far more abundant than other species within a size bin, or because they are known to live in a different part of the water column from another species. In some cases, we could only say that a certain size bin contained one or more of a several species of similar size. Where there were high concentrations of zooplankton or other large particles, it was not possible to differentiate taxa because coincidence often caused species to appear larger than they really were and shifted biomass to larger size bins (Liebig et al. 2006). This is a bigger problem in Lake Erie than Lake Michigan.

The bin divisions for the Lake Michigan example were determined based on the data shown in Figure 1. The 0.5 mm division was designated in order to separate *Leptodiatomus sicilis* from smaller copepods. Based on the net sample data from our example site, it may have been better to place that division at about 0.4 mm because then it would include all or most calanoid copepods as well as all or most of the small *Daphnia*. We were trying to make the bin sizes generic for a lake, but depending on how much custom data processing you want to do and how much change there is over the length of a transect, bin sizes could be custom fit to a site or transect.

The following is a summary of criteria necessary to apply zooplankton species names or taxonomic groups to OPC data:

- Little or no OPC coincidence.
- Lengths and corresponding ESDs of species in study area.
- Good size separation between at least some of the species present.
- Knowledge of zooplankton species composition and relative abundance from net samples at a particular site to determine the significance and dominance of particular taxa.
- Knowledge of a species range in habitat (i.e. where they are usually found in the water column and whether they live inshore or offshore) to determine spatial separation.

In addition to knowing ESD/L ratios for choosing bin sizes for zooplankton, these ratios are useful for converting L to ESD, which may be a better measure of zooplankton conspicuousness, in regard to predators, than length. ESD also has the attractive feature of being easily related to mass (volume).

5. ACKNOWLEDGEMENTS

We thank the crews of the R/V *Laurentian* and the R/V *Lake Guardian* for assistance in field operations. We thank M. Bundy for use of the OPC-1L and T. Miller for assistance in constructing the OPC circulator system. We also thank J. Cavaletto and M. Clouse for analyzing zooplankton samples and G. Lang for processing PSS field output.

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