Methods for the Determination of Zooplankton Density, Biomass and Secondary Production

K.L. Bowen

Great Lakes Laboratory for Fisheries and Aquatic Sciences,
Central and Arctic Region, Fisheries and Oceans Canada,
867 Lakeshore Road, Burlington, ON, L7S 1A1

2017

Canadian Manuscript Report of
Fisheries and Aquatic Sciences 3119

Fisheries and Oceans Canada | Pêches et Océans Canada

Canada

Canadian Manuscript Report of Fisheries and Aquatic Sciences

Manuscript reports contain scientific and technical information that contributes to existing knowledge but which deals with national or regional problems. Distribution is restricted to institutions or individuals located in particular regions of Canada. However, no restriction is placed on subject matter, and the series reflects the broad interests and policies of Fisheries and Oceans Canada, namely, fisheries and aquatic sciences.

Manuscript reports may be cited as full publications. The correct citation appears above the abstract of each report. Each report is abstracted in the data base *Aquatic Sciences and Fisheries Abstracts*.

Manuscript reports are produced regionally but are numbered nationally. Requests for individual reports will be filled by the issuing establishment listed on the front cover and title page.

Numbers 1-900 in this series were issued as Manuscript Reports (Biological Series) of the Biological Board of Canada, and subsequent to 1937 when the name of the Board was changed by Act of Parliament, as Manuscript Reports (Biological Series) of the Fisheries Research Board of Canada. Numbers 1426 - 1550 were issued as Department of Fisheries and Environment, Fisheries and Marine Service Manuscript Reports. The current series name was changed with report number 1551.

Rapport manuscrit canadien des sciences halieutiques et aquatiques

Les rapports manuscrits contiennent des renseignements scientifiques et techniques qui constituent une contribution aux connaissances actuelles, mais qui traitent de problèmes nationaux ou régionaux. La distribution en est limitée aux organismes et aux personnes de régions particulières du Canada. II n'y a aucune restriction quant au sujet; de fait, la série reflète la vaste gamme des intérêts et des politiques de Pêches et Océans Canada, c'est-à-dire les sciences halieutiques et aquatiques.

Les rapports manuscrits peuvent être cités comme des publications à part entière. Le titre exact figure au-dessus du résumé de chaque rapport. Les rapports manuscrits sont résumés dans la base de données *Résumés des sciences aquatiques et halieutiques.*

Les rapports manuscrits sont produits à l'échelon régional, mais numérotés à l'échelon national. Les demandes de rapports seront satisfaites par l'établissement auteur dont le nom figure sur la couverture et la page du titre.

Les numéros 1 à 900 de cette série ont été publiés à titre de Manuscrits (série biologique) de l'Office de biologie du Canada, et après le changement de la désignation de cet organisme par décret du Parlement, en 1937, ont été classés comme Manuscrits (série biologique) de l'Office des recherches sur les pêcheries du Canada. Les numéros 901 à 1425 ont été publiés à titre de Rapports manuscrits de l'Office des recherches sur les pêcheries du Canada. Les numéros 1426 à 1550 sont parus à titre de Rapports manuscrits du Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom actuel de la série a été établi lors de la parution du numéro 1551.

Canadian Manuscript Report of

Fisheries and Aquatic Sciences 3119

2017

Methods for the determination of zooplankton density, biomass and secondary production

By

K.L. Bowen

Great Lakes Laboratory for Fisheries and Aquatic Sciences, Central and Arctic Region, Fisheries and Oceans Canada, 867 Lakeshore Road, Burlington, ON, L7S 1A1

Cat. No. Fs97-4/3119E-PDF ISBN 978-0-660-08553-1 ISSN 1488-5387

Correct citation for this publication:

Bowen, K.L. 2017. Methods for the determination of zooplankton density, biomass and secondary production. Can. Manu. Rep. Fish. Aquat. Sci. 3119: vii + 50p.

CONTENTS

ABSTRACT

This report details the methods used by DFO's Great Lakes Laboratory for Fisheries and Aquatic Sciences (GLLFAS) in Burlington, Ontario, Canada for zooplankton and rotifer sample splitting, counting and biomass determination. These enumeration methods have been in place since the 1970s to analyze samples collected in open water and embayments in the Laurentian Great Lakes, including Lake Erie, the Bay of Quinte and Hamilton Harbour. It includes standard operating procedures for sample counts, including subsampling techniques, the number of animals counted and measured, length-weight regressions and taxonomic references. This report also describes how to calculate secondary production using both the egg ratio (ER) and production/biomass (P/B) methods, and the utility and limitations of each. Although agreement between the two methods is often good, the P/B technique often overestimates herbivorous cladoceran and cyclopoid production relative to the ER method at our sites, but underestimates production of diaptomid copepods and the invasive predatory cladocerans *Cercopagis* and *Bythotrephes*. Calculating P/B production by *Dreissena bugensis* veligers is problematic due to the lack of growth rate information for this species. When egg data are insufficient to use the preferred ER method, correction equations are given to adjust estimates of P/B production at Great Lakes locations. These may have utility in improving secondary production estimates at other locations.

RESUMÉ

Ce rapport présente les méthodes utilisées par le Laboratoire des Grands Lacs pour les pêches et les sciences aquatiques (LGLPSA) du MPO à Burlington, Ontario, Canada, pour le fractionnement, le comptage et la détermination de la biomasse des zooplanctons et des rotifères. Ces méthodes d'énumération sont en place depuis les années 1970 pour analyser les échantillons prélevés dans les eaux de surface et les baies des Grands Lacs D'Amérique du Nord, y compris le lac Érié, la baie de Quinte et le port de Hamilton. Il comprend des procédures opérationnelles normalisées pour le comptage d'échantillons, y compris les techniques de sous-échantillonnage, le nombre d'animaux comptés et mesurés, les relations de longueur-poids et les références taxonomiques. Ce rapport décrit également comment calculer la production secondaire en utilisant à la fois les méthodes du ratio d'oeufs (ER) and de production / biomasse (P / B), ainsi que l'utilité et les limites de chacun. Bien que l'accord entre les deux méthodes soit souvent bon, la technique P / B surestime souvent la production des cladocères et des cyclopoïdes herbivores par rapport à la méthode ER à nos sites, mais sous-estime la production des copépodes diaptomidés et des cladocères prédateurs invasifs Cercopagis et Bythotrephes. Le calcul de la production P / B par les véligères de *Dreissena bugensis* est problématique en raison qu'il manque d'information sur le taux de croissance de cette espèce. Lorsque les données sur les oeufs sont insuffisantes pour utiliser la méthode préférée de ER, des équations de correction sont fournies pour ajuster les estimations de la production P / B dans les sites des Grands Lacs. Ceux-ci peuvent être utiles pour améliorer les estimations de la production secondaire à d'autres endroits.

1.0. INTRODUCTION

Accurate estimation of zooplankton densities, biomass and secondary production are essential to ecosystem studies in both freshwater and marine environments, particularly for those modelling trophic relationships and energy transfer. Quantifying zooplankton production is a key part of understanding ecosystem structure and function, especially following invasion by non-native species and changes in trophic status, phytoplankton and fish communities. However, sampling zooplankton in the field is notoriously fraught with error (Bottrell et al. 1976, Downing and Rigler 1984, Mack et al. 2012), due to the patchiness of zooplankton, net efficiency estimation and gear avoidance by the zooplankters. Following field collection, lab procedure errors include mistakes in counting methodology, subsampling variability and the use of inappropriate length-weight relationships for biomass estimates (Mack et al. 2012, McCauley 1984, Sell and Evans 1982). Even when carried out correctly, subtle differences in laboratory techniques may result in variable density and biomass estimates among taxonomists. It is important to understand the biases imparted by differences among sampling program methods and to standardize field and laboratory techniques as much as possible.

The purpose of this technical report is to describe in detail post-field collection methods employed by zooplankton researchers at DFO's Great Lakes Laboratory for Fisheries and Aquatic Sciences (GLLFAS) in Burlington, Ontario, Canada since the early 1990s. These include the description of a new sample splitting technique, detailed sample enumeration methods and length-weight regressions used to estimate both macrozooplankton and rotifer biomass. It also describes allocation of loose eggs and juvenile copepods in samples, and presents the rationale and calculations used to determine zooplankton production using two methods: Egg Ratio (ER) and P/B. Finally, it compares production estimates obtained from these two methods for zooplankton collected from several Laurentian Great Lakes systems. Correction equations used to adjust estimates of P/B production at these locations are also presented, which may have utility in estimating secondary production at other locations.

2.0. ENUMERATION OF SAMPLES

2.1. ZOOPLANKTON SAMPLE SPLITTING

It is often necessary to accurately split preserved zooplankton samples into two or more subsamples prior to analysis. Splitting samples into smaller fractions for enumeration is usually necessary due to the large numbers of animals present in the original sample; however splitting of samples introduces another potential source of error (Bottrell et al. 1976). Splitting may also be done to create a back-up sample in case the primary sample is lost or destroyed during shipping or counting, or to send subsamples to multiple contractors. It is also sometimes necessary to create depth or seasonal composites if resources do not allow for the enumeration of individual date or depth stratum samples. For creating seasonal composites, it may be sufficient to split the sample into two equal parts if the volume collected on each date was the same (e.g.,

Schindler Patalas traps). However, if vertical net hauls were used, the sample volume collected may be different on each date due to differences in stratum depth or net efficiency. As each sampling date should be equally represented in the seasonal composite (e.g., the equivalent of 100 L), the fraction of the sample used to make the composite may be different for each date.

The Folsom plankton splitter is often used for splitting samples into two equal parts (Sell and Evans 1982, McEwen et al. 1954, Van Guelpen et al. 1982). However, its use can be cumbersome and messy, especially when working with sugar-formalin preserved samples. Dividing samples into smaller fractions requires time-consuming repeated splitting, which can also introduce error (Lee and McAlice 1979, McEwen et al. 1954). As a result of these limitations, we have developed a syringe-based method of splitting samples into equal or unequal fractions. This employs a 50 mL plastic syringe with the tip removed, resulting in an opening about 5 mm in diameter. The agitated sample is poured into a calibrated beaker and the volume increased to a known amount using water or preservative (e.g., 100 mL). Immediately after pouring, the sample is randomly mixed using the syringe and the desired amount is drawn into the syringe barrel. Random mixing (e.g., a figure 8 pattern or a “plus” pattern with the syringe) is necessary, as mixing the sample in a circular pattern moves the particulates into the middle of the container and skews results. The removed fraction can then easily be added to a narrow-necked jar such as a flint archive bottle, or combined with fractions from other samples to make a seasonal or depth composite. The syringe should be rinsed with a small amount of water or preservative to remove any animals adhering to the inside of the barrel. We have found this method to be quick and clean, and it allows the removal of variable sample fractions that might be required when making composites. Occasionally applying a small amount of silicone lubricant to the rubber seal on the plunger is helpful. Although the syringe opening has been enlarged to prevent clogging, large animals such as *Bythotrephes* or *Mysis* should be removed prior to splitting. Clogging of the syringe may also be problematic in samples containing large fragments of detritus or macrophytes. Conversely, for samples containing only very small organisms and little debris, the syringe tip may be retained. Attaching a short piece of tubing to the tip allows the removal of known fractions from narrow-necked archive jars, following the agitation of the sample.

To test the efficacy of the syringe method for splitting a sample into two equal parts, three offshore zooplankton samples collected from Lake Ontario in September 2014 were tested. Zooplankton densities in these samples ranged from 33.4 to 282 animals L^{-1}, and were dominated by microzooplankton (veligers and copepod nauplii). Three subsamples were taken from each of the “removed” and “remaining” fractions using a 1 mL Hensen-Stempel pipette, and the number of *Dreissena* veligers, copepod nauplii, *Daphnia* and cyclopoids (adults and copepodids) were counted in each using a 6 mL Bogorov counting chamber and a dissecting microscope (See Appendix 1 for details). Counting individual subsamples provides information on subsampling variability using the Hensen-Stempel pipette. There were no significant differences between the “removed” or “remaining” fractions for any group (ANOVA, $p>0.05$; Figure 1). This demonstrates that the syringe technique splits zooplankton samples

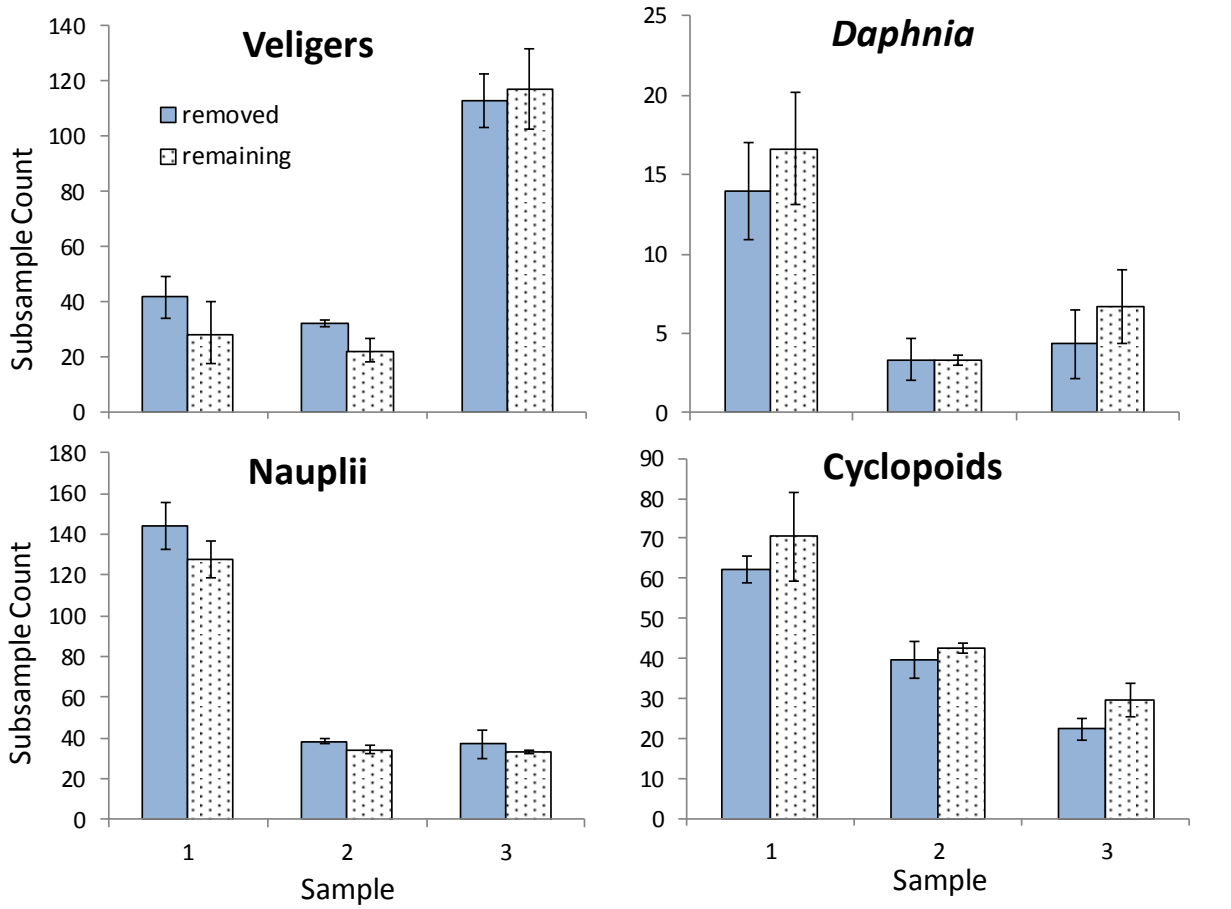


Figure 1. Counts (±SE) of veligers, *Daphnia*, copepod nauplii and cyclopoids (adults and copepodids) from the "syringe splitting" experiment where three September 2014 Lake Ontario samples were split into two equal parts ("removed" with the syringe and "remaining" in the beaker). For each sample, three subsamples were enumerated from each half using a Hensen-Stempel pipette.

equally for small, dense animals that settle quickly such as veligers, and for larger zooplankton such as *Daphnia*. However, it is likely not effective in splitting taxa that easily tangle into clumps such as the fish-hook water flea *Cercopagis pengoi* (hereafter *Cercopagis*).

2.2. CERCOPAGIS AND BYTHOTREPHES ENUMERATION

Two invasive predatory cladocerans, *Cercopagis* and the spiny waterflea *Bythotrephes longimanus* (hereafter *Bythotrephes*) are often of special interest in limnological surveys given their ecological impacts on the zooplankton community in many invaded systems (Strecker and Arnott 2008, Warner et al. 2006). *Cercopagis* cannot be accurately counted using subsampling techniques because individuals form clumps in the preserved samples as their caudal spines become entangled. Although *Bythotrephes* is

not as likely to form clumps, they are very large but often rare and may not be accurately enumerated if only a small fraction of the sample is counted. Prior to any splitting or pooling of samples, the entire preserved sample is strained through a 400-*μ*m mesh sieve to remove algae and smaller organisms, and the entire large fraction is rinsed with water and scanned for *Cercopagis* and *Bythotrephes* (See Appendix 1 for details). The small and large fractions are then recombined and re-preserved for further taxonomic analyses.

For each of these two species, up to 30 random individuals per sample were measured (Figure 2) to determine lengths and the number of eggs or embryos present and their development stage. Length-weight regression equations were used to calculate dry weight of each individual (Table 1). This sample size provides reliable estimates of both mean lengths and mean numbers of eggs without wasting counting effort. This was determined by subsampling *Cercopagis* measurements and egg counts taken on four separate dates at Conway in the Bay of Quinte, where at least 50 individuals were measured on each date (Figure 3).

Large clumps of *Cercopagis* entangled with algae, detritus and other zooplankton may be present in the samples. As these clumps may contain hundreds or thousands of individuals, it is prohibitively time consuming to count all of them. Any large clumps found in a sample should be removed and pooled in a petri dish with water. The number of individuals present can be estimated by removing a portion of the clump and counting the number of *Cercopagis* it contains (ideally 50 to 150 animals). Both the smaller, counted portion and the remaining clump, including associated algae and detritus, are blotted dry on a piece of lab tissue. The blotted clumps are then weighed on a scientific balance accurate to 0.01 mg. Mean mass per individual is determined, and the mass of the remaining clump is divided by this value to determine the total number of *Cercopagis* present. As this mass per individual also contains associated detrital material, it should not be used as an accurate estimation of the animal's wet weight in further analyses.

2.3. SAMPLE ENUMERATION – ZOOPLANKTON

Starting in fall 2014, technicians with GLLFAS in Burlington ON developed an "in-house" zooplankton counting procedure, to be used for selected samples that do not require a high degree of taxonomic precision (details in Appendix 1). For samples analyzed by experienced zooplankton taxonomists outside of GLLFAS, the standard counting guidelines have been in place since the 1970s, and are outlined in the following paragraphs. Although similar to those in Appendix 1, the guidelines for outside taxonomists generally require greater taxonomic resolution. These state that a minimum of 400 individual zooplankton are to be enumerated using a stratified, random, counting procedure (Cooley et al. 1986) ensuring that at least 100 individuals of each major group are included. For samples dominated by only one species, a minimum of 200 individuals are counted. If animal density is low, up to 20% of the sample is to be counted. However, any subsamples taken for enumeration must be counted in their

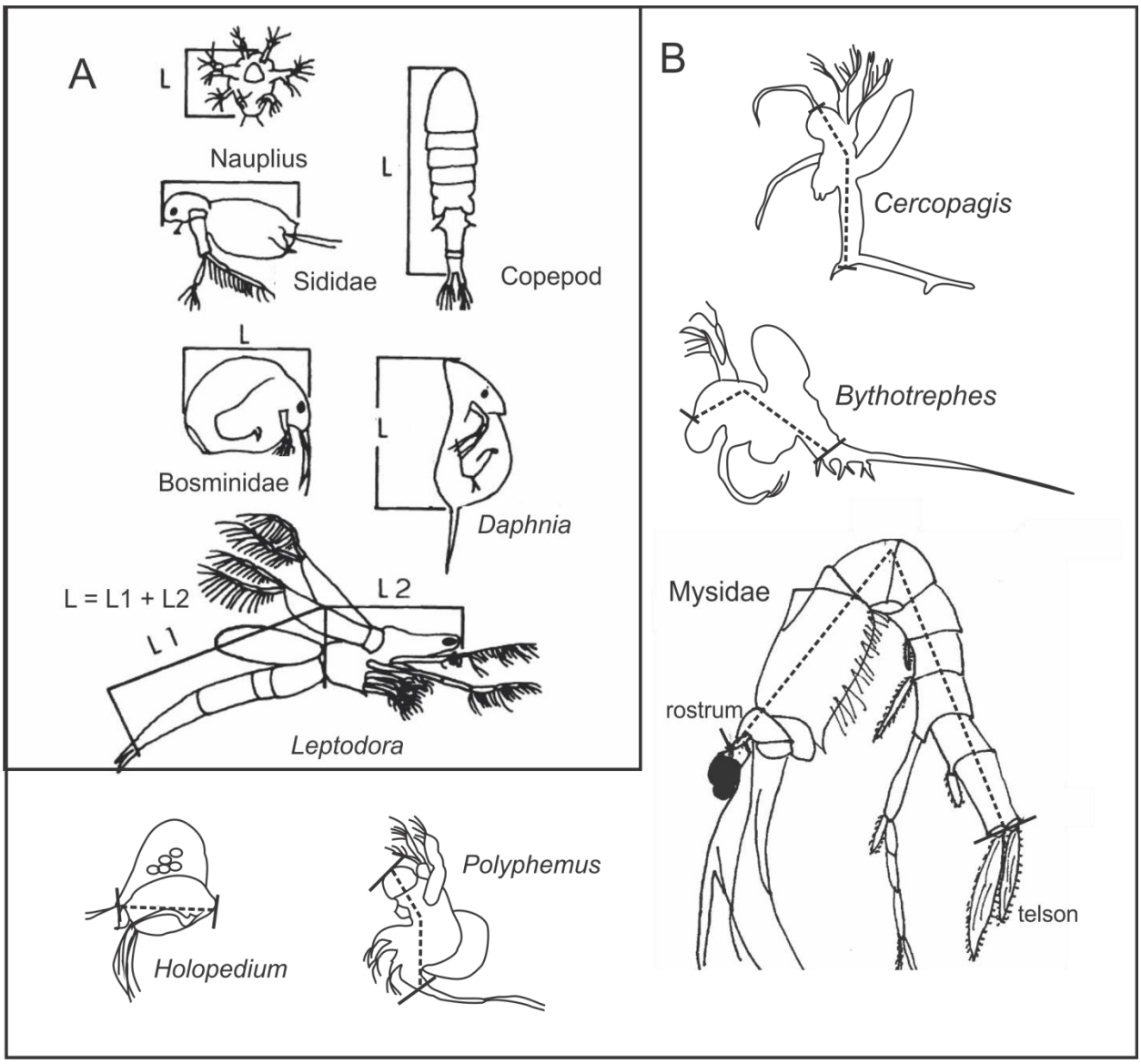


Figure 2. Measuring locations to be used when determining length (L) of various zooplankton taxa. A) is taken from Culver et al. 1985. B) Other taxa where dotted line shows measuring path.

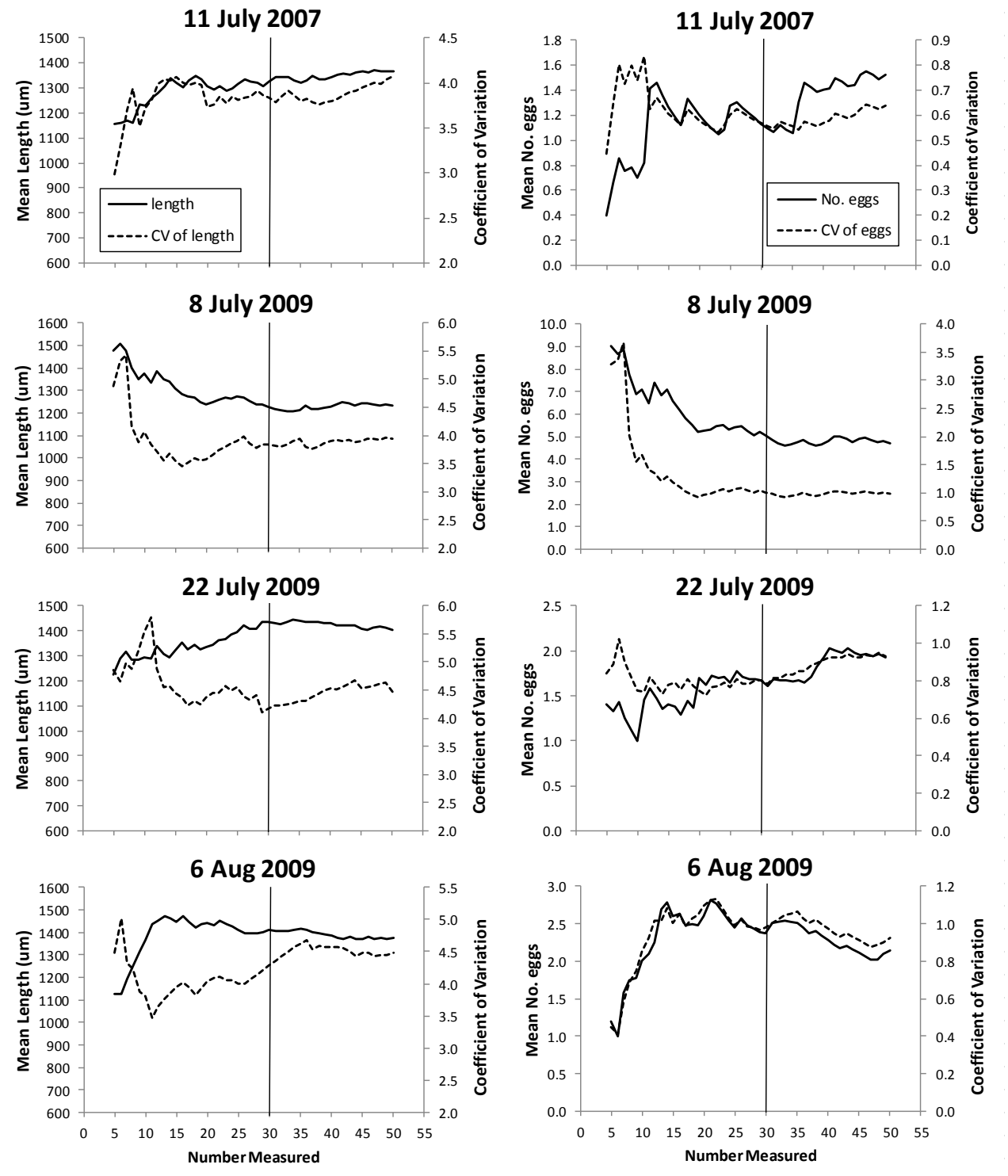


Figure 3. Effect of sample size on mean *Cercopagis* length, mean number of eggs per animal and coefficient of variation (CV) on four dates at Conway in the Bay of Quinte. The vertical line on each graph indicates 30 animals, the number chosen for measurement. This is the point at which the means generally stabilize and accuracy does not tend to improve with further effort.

entirety. Cladocerans and adult cyclopoids and calanoids are identified to species, the nauplii and copepodids to order and *Dreissena* mussel larvae as veligers. *Limnocalanus* and *Epishura* copepodids are identified to species. As there is taxonomic uncertainty within the genus *Bosmina* in the Great Lakes, this taxon is usually only identified only to genus. Taxonomic references are provided in Appendix 2.

Depending on their abundance, lengths are measured for up to 30 individuals for veligers and each type of nauplii and copepodids, and up to 50 individuals for each cladoceran and copepod identified to species. Measurements are done manually using a dissecting microscope equipped with a camera lucida and SummaSketch III digitizing system, or with a digital camera and imaging software connected to a spreadsheet. Lengths of herbivorous cladocerans are measured from the top of the helmet to the base of the tail spine, copepods from the anterior end of the cephalothorax to the end of the caudal rami, and veligers across the widest section of the shell (Figure 2). Dry weights are estimated from length-weight regressions obtained from the literature (Table 1). These are calculated from each individual length and then averaged to determine a mean weight for each taxon.

The number of eggs carried by each measured adult individual and the number of loose eggs are to be determined for the following categories: cladoceran, cyclopoid, calanoid and unidentified copepod. Cladoceran neonates too small to be living independently are included in the loose egg count. Because production is estimated from these data by the egg ratio method, sufficient emphasis is to be placed on enumeration of adult stages of dominant copepod species even when the sample is dominated by immature forms. Eggs are not measured, nor should they constitute part of the 400 animal totals. A maximum of 100 clustered eggs (copepods) and 100 loose eggs are to be counted.

Once these goals have been met, the remainder of the animals within the counting cell (e.g., Bogorov chamber, Ward counting wheel) should be enumerated but not measured. Enumerating only part of a chamber may result in a biased count as the subsample may not be evenly distributed throughout the chamber. Care should be taken to obtain a proper dilution of the sample prior to removing subsamples with a device such as a Hensen-Stempel pipette. This will ensure that it is feasible to count all of the animals contained within the subsample. Several subsamples may be required to reach the counting goals outlined above.

To determine the amount of subsampling error associated with this counting method, 10 zooplankton samples collected in 2008 and 2009 at each of two stations in the Bay of Quinte were recounted by the same taxonomist. When the total zooplankton density obtained by the original count for each sample was compared to the recount, the mean coefficient of variation (CV) was 0.12 for Belleville and 0.20 for Conway. When the two stations were combined, there were no significant differences ($p>0.05$) between the original and recounted values for total zooplankton when tested using a two-tailed paired t-test ($t=2.09$; $N=20$; $p=0.75$). When groups were examined individually, there were also no differences for herbivorous cladocerans, cyclopoids, calanoids or veligers.

Table 1. Length-weight relationships used to estimate zooplankton biomass modified from Johannsson et al. (2000). Unless specified otherwise, $W = aL^b$, where L is length in mm and W is dry weight in µg.

Taxon	a	b	Source
MYSIDS*			
Mysis diluviana	4.67	2.72	Johannsson (1995)
Hemimysis anomala	6.48	2.75	Marty et al. (2011)
CLADOCERA			
Bosmina, Eubosmina	10.72	2.12	Bowen and Johannsson (2011)
Daphnia, Diaphanosoma, Sida	5.00	2.84	Bottrell et al. 1976 (Dumont et al. 1975)
Polyphemus pediculus	6.93	2.15	Dumont et al. (1975)
Holopedium gibberum	11.21	3.04	Yan (OMEE, Dorset, pers. com.)
Chydorus sphaericus	33.23	3.21	Malley et al. (1989)
Alona sp.	29.70	3.48	Dumont et al. (1975)
Bythothrephes longimanus	11.13	2.77	Yan (OMEE, Dorset, pers. com.)
*Cercopagis pengoi***	0.38	2.44	Grigorovich et al. (2000)
Leptodora kindti	0.44	2.67	Rosen (1981)
COPEPODA			
CALANOIDA			
Generic equation	5.50	2.46	Sprules (U. of Toronto, pers. com.)
Epischura	6.50	2.63	Culver et al. (1985)
Senecella calanoides	7.70	2.33	Culver et al. (1985)
Calanoid nauplii	4.20	2.48	Sprules (U. of Toronto, pers. com.)
CYCLOPOIDA			
Generic Equation	5.50	2.46	Sprules (U. of Toronto, pers. com.)
Mesocyclops edax	6.66	2.89	Culver et al. (1985)
Cyclopoid nauplii	4.20	2.48	Sprules (U. of Toronto, pers. com.)
HARPACTACOIDA	4.20	2.48	Sprules (U. of Toronto, pers. com.)
DREISSENA* VELIGERS**	0.025	0.018	Bowen et al. (in press)

* Mysid length measurements exclude the telson (as seen in Figure 2)
** Biomass of *Cercopagis pengoi* is determined using the equation logW=a+b log(L).
*** Biomass of *Dreissena* is $W = a\,e^{bL}$ where L is length in µm and W is dry weight in µg.

To determine the amount of subsampling error associated with this counting method, 10 zooplankton samples collected in 2008 and 2009 at each of two stations in the Bay of Quinte were recounted by the same taxonomist. When the total zooplankton density obtained by the original count for each sample was compared to the recount, the mean coefficient of variation (CV) was 0.12 for Belleville and 0.20 for Conway. When the two stations were combined, there were no significant differences ($p>0.05$) between the original and recounted values for total zooplankton when tested using a two-tailed paired t-test ($t=2.09$; $N=20$; $p=0.75$). When groups were examined individually, there were also no differences for herbivorous cladocerans, cyclopoids, calanoids or veligers.

2.4. SAMPLE ENUMERATION – ROTIFERS

All rotifer samples are collected in the field by filtering whole water samples through a 20 µm mesh sieve. This fine mesh better captures small rotifers that may otherwise be lost through standard 64 µm nets (Bottrell et al. 1976, Chick et al. 2010). Therefore it is important to use 20 µm mesh sieves in all laboratory procedures involving rotifers. One way to subsample rotifers for enumeration is to thoroughly mix the sample by gentle bubbling (Johannsson et al. 2000). Subsamples are removed by syringe to a Sedgewick-Rafter chamber for counting, using the stratified, random counting procedure of Cooley et al. (1986) described in Section 2.3. Although the goal is to identify and measure 400 animals, examine a maximum of 25% of the sample by volume if the biomass is low. Measure 30 of each type for common individuals, and for rare animals, as many as are encountered in the chamber. If counting must continue after these goals are met to complete the minimum volume in the counting chamber, animals do not need to be measured. Wet weight for each individual is calculated by using the biovolume formulae of Ruttner-Kolisko (in McCauley, 1984), or Johannsson et al. (2000) for *Polyarthra* (Table 2). Dry weights are calculated by multiplying wet weight by 0.1, or in the case of *Asplanchna* sp. by 0.036 (Dumont et al., 1975). Mean rotifer wet weights for various Great Lakes systems sampled as part of DFO monitoring programs are also given in Table 2, with station locations in Appendix 3. These include Lake Erie in 1993 and 1994 (Johannsson et al. 2000), and sites in Lake Ontario, Hamilton Harbour, Bay of Quinte and open water areas of Lake Huron sampled between 2000 and 2015.

3.0. ZOOPLANKTON PRODUCTION ESTIMATES

3.1. ALLOCATION OF JUVENILE COPEPODS

Production estimates for each copepod taxon use the total density of adults plus the density of juveniles (copepodids and nauplii) belonging to that taxon. As these juveniles are generally just identified to order (cyclopoid or calanoid), they need to be proportionally allocated to each taxon based on adult density. *Limnocalanus* and *Epischura* copepodids are usually identified to species, so these two taxa are not included in this allocation process. Our laboratory uses a customized computer program “PGLLFAS” written by Jim Moore to perform the following allocations. For a given order, the proportion of each adult species relative to the total density of adults is determined

Table 2. Length weight regression equations for common rotifer taxa. Mean wet weights are from DFO Great Lakes sampling programs. Equation Type 1 is $ww = aL^3 \times 10^{-6}$ and Type 2 is $ww = aLW^2 \times 10^{-6}$; where ww is wet weight in µg, L is length (longest dimension) in µm and W is width in µm. Formulae are from Ruttner-Kolisko (in McCauley, 1984), with the exception of *Polyarthra* (Johannsson et al. 2000). Dry weights are obtained by multiplying wet weights by 0.1, or 0.036 for *Asplanchna*.

				Mean Wet Weights (µg)					
Genus	**Species**	**Eq'n**	***a***	**Quinte**	**Ham.**	**Erie**	**Huron**	**Ontario**	**mean**
Anuraeopsis	*fissa*	1	0.030	0.023	-	-	0.016	0.014	0.017
Ascomorpha	*ovalis*	1	0.120	0.102	0.111	0.191	0.106	0.096	0.114
Ascomorpha	sp.	1	0.120	0.100	0.112	-	0.092	0.078	0.087
Asplanchna	*priodonta*	1	0.230	29.32	17.05	23.51	19.61	28.88	23.98
Asplanchna	sp.	1	0.230	28.59	20.44	34.00	14.21	25.25	23.64
Brachionus	*angularis*	1	0.120	0.920	0.368	0.185	-	0.220	0.302
Conochilus	*unicornis*	2	0.260	0.130	0.117	0.252	0.148	0.140	0.173
Euchlanis	sp.	1	0.100	1.515	-	0.196	-	1.217	1.353
Filinia	*brachiata*	1	0.130	0.067	-	-	-	-	0.067
Filinia	*longiseta*	1	0.130	0.113	0.147	0.464	0.186	0.141	0.303
Filinia	*terminalis*	1	0.130	0.140	0.269	-	0.159	0.207	0.226
Gastropus	*stylifer*	1	0.200	0.186	0.248	0.289	0.162	0.226	0.228
Kellicottia	*longispina*	1	0.030	0.053	0.054	0.095	0.052	0.060	0.069
Keratella	*cochlearis*	1	0.020	0.039	0.041	0.043	0.044	0.056	0.046
Keratella	*cochlearis tecta*	1	0.020	0.009	0.011	-	-	0.012	0.011
Keratella	*quadrata*	1	0.220	0.583	0.616	0.576	0.533	0.651	0.605
Keratella	*serrulata*	1	0.020	0.012	0.011	-	-	0.012	0.012
Notholca	*squamula*	1	0.035	0.047	0.031	0.206	0.029	0.120	0.130
Ploesoma	*hudsoni*	1	0.100	2.168	2.256	2.576	1.251	1.429	1.691
Ploesoma	*truncatum*	1	0.100	0.305	0.408	0.353	0.215	0.233	0.295
Polyarthra	*dolichoptera*	1	0.205	0.087	0.113	0.183	0.088	0.095	0.115
Polyarthra	*major*	1	0.158	0.362	0.404	0.537	0.271	0.246	0.440
Polyarthra	*remata*	1	0.158	0.033	0.030	0.105	0.030	0.031	0.068
Polyarthra	*vulgaris*	1	0.158	0.131	0.147	0.256	0.118	0.101	0.167
Pompholyx	*sulcata*	1	0.150	0.114	0.119	-	0.151	0.114	0.118
Synchaeta	*grandis*	1	0.100	0.955	1.758	-	-	2.043	1.891
Synchaeta	*kitina*	1	0.100	0.091	0.100	0.067	0.073	0.097	0.092
Synchaeta	*pectinata*	1	0.100	0.430	0.715	0.259	0.292	0.499	0.499
Synchaeta	sp.	1	0.100	0.197	0.814	0.109	0.236	0.261	0.206
Synchaeta	*stylata*	1	0.100	0.517	0.922	0.732	0.365	0.549	0.549
Trichocerca	*cylindrica*	2	0.520	1.071	1.311	1.272	0.950	1.004	1.094
Trichocerca	*elongata*	2	0.520	1.273	1.527	1.068	1.264	1.239	1.246
Trichocerca	*longiseta*	2	0.520	0.179	0.176	-	0.187	0.178	0.178
Trichocerca	*multicrinis*	2	0.520	0.549	0.654	0.833	0.708	0.623	0.661
Trichocerca	*porcellus*	2	0.520	0.211	0.101	0.423	0.088	0.080	1.444
Trichocerca	*pusilla*	2	0.520	0.110	0.072	0.138	0.064	0.072	0.091
Trichocerca	*rousseleti*	2	0.520	0.074	0.053	0.089	0.059	0.066	0.071
Trichocerca	*similis*	2	0.520	0.178	0.169	0.235	0.272	0.184	0.196
Tylotrocha	*monopus*	1	0.035	0.066	0.081	0.208	0.064	0.047	0.098

in each sample, and juveniles are allocated by multiplying that proportion by the densities of both copepodids and nauplii. If there are no adults present on a given date, the proportions of adults from the previous and following sampling dates are averaged.

Mean copepod weights used for determination of biomass in egg ratio production calculations also must take juveniles into account. This is done as follows for each species on each date, where W = mean weight and D = density:

$$\frac{(copepodid\ W\ X\ copepodid\ D + nauplii\ W\ X\ nauplii\ D + adult\ W\ X\ adult\ D)}{\text{total D (incl.juv.)}} \quad \text{[eqn 1]}$$

Sometimes it may be necessary to calculate copepod production only to the order level, for example cases where copepods were only identified as cyclopoids or calanoids. Using these broader taxonomic groups for production estimates may also be desirable as it simplifies the allocation of both eggs and juveniles. For samples where nauplii are not identified to order, they can simply be divided among calanoids and cyclopoids based adult densities as described above. Another approach that takes reproductive seasonal patterns of each group into account is to use the average monthly proportions of calanoid and cyclopoid nauplii. These means were calculated from samples collected with 64 µm mesh in a variety of Great Lakes systems (Table 3), in which the nauplii were identified to order by trained taxonomists. Locations include the Bay of Quinte (1995 to 2016), Hamilton Harbour (2002 to 2016), LO81 in Lake Ontario (2007 -2016), Lake Huron (2007) and Lake Erie (1993, 1994 and 1998) (Appendix 3). The proportions of cyclopoid and calanoid nauplii will vary among aquatic systems, depending on the abundance and reproductive patterns of adult copepods. For example, cyclopoid nauplii tend to comprise 80 to 90% of the total nauplii density in Lake Ontario compared to about 60% in Lake Erie and only 5% in Lake Huron.

3.2. ALLOCATION OF LOOSE EGGS

Loose eggs (cladoceran, cyclopoid, calanoid and undetermined copepod) also need to be allocated to egg-bearing adults for egg ratio egg ratio production estimates (described in section 3.3). Loose undetermined copepod eggs may be assigned as either calanoid or cyclopoid based on the proportions of egg bearing adult copepods in the sample. However, if there are few egg-bearing adult copepods present, the average proportions of calanoid eggs compared to cyclopoid eggs for various systems may be used (Table 3). As with nauplii, these proportions vary both spatially and seasonally, and mirror the ratios of adult cyclopoids to calanoids in the system.

If copepod egg ratio production estimates are to be carried out for individual taxa, it is necessary to further assign cyclopoid or calanoid loose eggs to the taxa in each group. We use the PGLLFAS program for these allocations based on the proportion of attached egg densities in each taxon. For example, if measured *Daphnia retrocurva* contained a total of 20 eggs within their carapaces, and *Bosmina* had a total of 10 eggs, two thirds of loose cladoceran eggs would be assigned to *D. retrocurva*, and one third to *Bosmina*. Undetermined copepod eggs are assigned, if possible, to either or both cyclopoid and calanoid species based on egg densities. If there were no adults bearing

Table 3: Monthly proportions (by density in 64 µm mesh samples) of copepod nauplii and total eggs (loose plus attached) divided among cyclopoids and calanoids at stations in the Bay of Quinte (1995 -2016), Hamilton Harbour (2002 -2016), Lake Ontario (2007 - 2016), Lake Huron (2007) and Lake Erie (west and central-east in 1993, 1994 and 1998). The annual values are calculated by dividing the total number of eggs or nauplii over the sampling season in each group by the total number of both groups combined.

		Proportion of Total								
		Apr	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**annual**
Nauplii										
Quinte - B	cyclopoid	1.00	0.98	0.87	0.90	0.90	0.90	0.87	NA	**0.90**
	calanoid	0.00	0.02	0.13	0.10	0.10	0.10	0.13	NA	**0.10**
Quinte - C	cyclopoid	NA	0.93	0.87	0.93	0.85	0.72	0.77	NA	**0.84**
	calanoid	NA	0.07	0.13	0.07	0.15	0.28	0.23	NA	**0.16**
Hamilton	cyclopoid	1.00	0.98	0.89	0.87	0.84	0.79	0.74	0.53	**0.91**
	calanoid	0.00	0.02	0.11	0.13	0.16	0.21	0.26	0.47	**0.09**
Ont. - LO81	cyclopoid	0.57	0.82	0.95	0.97	0.76	0.72	0.66	0.32	**0.81**
	calanoid	0.43	0.18	0.05	0.03	0.24	0.28	0.34	0.68	**0.19**
Huron	cyclopoid	NA	0.08	NA	0.01	0.01	NA	0.10	NA	**0.05**
	calanoid	NA	0.92	NA	0.99	0.99	NA	0.90	NA	**0.95**
Erie West	cyclopoid	0.47	0.52	0.58	0.63	0.61	0.56	0.51	0.59	**0.58**
	calanoid	0.53	0.48	0.42	0.37	0.39	0.44	0.49	0.41	**0.42**
Erie CE	cyclopoid	0.50	0.67	0.77	0.54	0.56	0.55	0.57	0.92	**0.59**
	calanoid	0.50	0.33	0.23	0.46	0.44	0.45	0.43	0.08	**0.41**
Total Eggs										
Quinte - B	cyclopoid	1.00	1.00	0.83	0.85	0.90	0.97	0.70	NA	**0.90**
	calanoid	0.00	0.00	0.17	0.15	0.10	0.03	0.30	NA	**0.10**
Quinte - C	cyclopoid	NA	0.92	0.63	0.88	0.76	0.77	0.75	NA	**0.81**
	calanoid	NA	0.08	0.37	0.12	0.24	0.23	0.25	NA	**0.19**
Hamilton	cyclopoid	0.99	0.96	0.71	0.75	0.66	0.68	0.49	NA	**0.79**
	calanoid	0.01	0.04	0.29	0.25	0.34	0.32	0.51	NA	**0.21**
Ont. - LO81	cyclopoid	0.90	0.55	0.93	0.86	0.78	0.64	0.56	0.00	**0.73**
	calanoid	0.10	0.45	0.07	0.14	0.22	0.36	0.44	1.00	**0.27**
Huron	cyclopoid	NA	0.11	NA	0.33	0.29	NA	0.15	NA	**0.18**
	calanoid	NA	0.89	NA	0.67	0.71	NA	0.85	NA	**0.82**
Erie West	cyclopoid	0.36	0.33	0.63	0.71	0.26	0.54	0.45	0.48	**0.58**
	calanoid	0.64	0.67	0.37	0.29	0.74	0.46	0.55	0.52	**0.42**
Erie CE	cyclopoid	0.17	0.78	0.79	0.58	0.48	0.48	0.51	0.97	**0.61**
	calanoid	0.83	0.22	0.21	0.42	0.52	0.52	0.49	0.03	**0.39**

eggs in the sample for a given group, candidate species for assignment would include species in the sample for the week previous and week following the sample containing the loose eggs.

This process assumes that species lose eggs equally upon collection and preservation, and difficulties arise if very few individuals contain eggs but loose egg densities are high. For example, if only one copepod individual retained its egg sacs in a counted fraction, then all the loose eggs in the sample will be assigned to that species. This may result in a biased egg allocation. Sometimes only loose eggs are found in the sample, as seen in copepods collected in the Kingston Basin (LO81) of Lake Ontario in April (Table 4). When averaged over the season, the proportion of both cyclopoid and calanoid loose eggs may exceed 50% of the total eggs found (Table 4).

When egg-bearing individuals are rare in the counted sample, a better approach may be to assign eggs to each taxon based on the time of year and the known breeding season for common species. Monthly distributions of eggs among the dominant taxa are given in Table 4 for the epilimnion of LO81 in Lake Ontario. It would be reasonable to assign Lake Ontario cyclopoid eggs to *Diacyclops* in May and June, and divide eggs equally between *Diacyclops* and *Tropocyclops* for the remainder of the year. In terms of calanoids, *Leptodiaptomus sicilis* is the most common egg-bearing species in April and May, and tends to finish breeding by August. In June and July, eggs are more evenly distributed among the common calanoid species; *Eurytemora* tends to breed between July and October and *Skistodiaptomus oregonensis* between August and November. It is also noteworthy that *Limnocalanus* and *Epischura* breed outside of the sampling season and/or do not carry their egg sacs, and as a result no gravid females of these species have been found in any of our samples. Furthermore, as species composition and the seasonality of reproduction may vary in other systems, the ratios of eggs among dominant species may need to be determined for each system to properly allocate loose eggs.

3.3. EGG RATIO PRODUCTION METHOD

Zooplankton secondary production, the amount of biomass that is produced over the growing season, varies with taxonomic group, body size, population biomass, temperature and food availability (Humphreys 1979, Huntley and Lopez 1992, Plante and Downing 1989, Shuter and Ing 1997). Body size of individuals can be influenced by selective fish predation pressure on larger individuals (Almond et al. 1996), and gravid females in particular may be more conspicuous to visual predators. When reliable egg counts are available for zooplankton samples, the egg ratio (ER) method (Paloheimo 1974) is the preferred technique to calculate zooplankton production as it is based on actual egg densities rather than production rates obtained from the scientific literature. However, it may not yield accurate production estimates for uncommon taxa for which only a few individuals are usually measured. Deciding which taxa are abundant enough to use the ER method depends on measuring adequate numbers of individuals per taxon in each sample (e.g. >20 animals per date) during periods of peak abundance, rather than the densities or biomass of animals at that location. Based on our

Table 4. Percentage of zooplankton eggs associated with each taxonomic group for preserved epilimnetic sample collected at LO81 in the Kingston Basin, Lake Ontario. Samples were taken from 2007 to 2015 using vertical net hauls towed by hand.

	Percentage								
	Apr	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**mean**
Herbivorous Cladocerans*									
Bosmina	100	57	84	72	10	2	4	NA	32
Ceriodaphnia	0	0	1	5	3	2	0	NA	4
Chydorus	0	1	1	1	6	17	2	NA	6
D. galeata	0	0	2	1	2	14	6	NA	4
D. retrocurva	0	14	5	10	30	15	4	NA	19
Diaphanosoma	0	0	0	0	1	0	0	NA	1
Eubosmina	0	28	3	4	19	44	36	NA	15
Holopedium	0	0	4	7	29	7	48	NA	19
Cyclopoids*									
Diacyclops	NA	98	100	31	58	51	36	NA	74
Mesocyclops	NA	0	0	0	0	0	0	NA	0
Tropocyclops	NA	2	0	69	42	49	64	NA	26
Calanoids*									
D. minutus	0	8	41	6	35	18	12	0	9
S. oregonensis	0	2	29	11	65	56	26	100	19
D. sicilis	100	89	30	37	0	0	3	0	60
D. siciloides	0	0	0	0	0	0	0	0	0
Epischura	0	0	0	0	0	0	0	0	0
Eurytemora	0	0	0	45	0	26	59	0	12
Limnocalanus	0	0	0	0	0	0	0	0	0
Percent Loose Eggs**									
Cladocera	0	18	9	12	16	20	17	0	14
Cyclopoida	100	35	79	65	75	86	38	NA	57
Calanoida	91	32	30	46	80	74	73	99	58

* includes only those eggs attached to individuals

** Mean percentage of loose eggs relative to the total number of eggs for that group (loose plus attached). Includes undetermined copepod eggs, which were allocated to cyclopoid or calanoid based on proportion of egg densities for each group. Herbivorous cladoceran loose eggs includes neonates lost from the brood pouches.

NA no attached eggs were found for that group

enumeration requirements, the fraction counted will simply be higher for low density samples. For example, in the Bay of Quinte (Johannsson and Bowen 2012) and Hamilton Harbour (Bowen and Currie 2017), zooplankton were often very abundant, with May to October areal biomass means frequently ranging between 1000 and 3000 mg m^{-2} or more. To ensure that adequate numbers were measured in these two studies, the ER method was only used for taxa with areal biomass >50 mg m^{-2} for any given station and year. At LO81 in the Kingston Basin of Lake Ontario, where zooplankton May to October areal biomass never exceeded 1000 mg m^{-2}, the ER method was used for cladocerans with biomass >2 mg m^{-2}. When the whole sample is enumerated for taxa such as *Cercopagis* and *Bythotrephes*, adequate numbers of individuals can often be measured to utilize the ER method even when densities are relatively low.

The ER method also cannot be used for taxa that shed their eggs upon fertilization such as the calanoids *Epischura* and *Limnocalanus* (Selgeby 1975). These taxa also tend to spawn during the winter months when zooplankton samples are typically not collected. In embayments that warm quickly in the spring such as Hamilton Harbour, some egg production by early spawning taxa such as the cyclopoid *Diacyclops* may be missed if sampling does not begin until May. The highest cyclopoid nauplii densities were observed in April in Hamilton Harbour (Bowen and Currie, 2017), suggesting that cyclopoid reproduction begins in the early spring. ER production values for cyclopoids relative to values based on the P/B method (Section 3.5) are lower in Hamilton Harbour than for any other system shown in Figure 4, supporting the idea that early spring egg production is being missed. The proportion of copepod reproduction that occurs during the infrequently-sampled November to March period is largely unknown. Excluding the winter period in annual production calculations (e.g., Johannsson and Bowen 2012; Bowen and Currie 2017) may lead to underestimates of total seasonal production, especially for winter or early spring spawning taxa. Winter is increasingly recognized as an important season for freshwater calanoids that remain active throughout the year (Vanderploeg et al. 1992, Kerfoot et al. 2008), although more research is needed on the extent of winter production for both phytoplankton and zooplankton.

Given the influence of temperature on zooplankton development times (Bottrell et al. 1976), it is desirable to calculate zooplankton production within individual thermal layers for the duration of each layer. For each species, the production values for each layer are then summed to generate total seasonal water column production. Calculations for ER production are given in Stockwell and Johannsson (1997) and are described in detail in Appendix 4. In summary, the ER method determines production for each taxon for each interval between sampling dates based on changes in density (including juveniles) and the average egg ratio, animal mass and temperature for that interval. The production estimates for each interval are added up to provide an estimate of total production across the sampling season. The egg ratio for a given taxon is the total egg density (including allocated loose eggs) in the sample divided by the density of individuals, which includes both adults and juveniles. Bělehrádek's coefficients used to calculate egg development times (K) for common taxa are given in Table 5, taken from Johannsson et al. (2000). However, this publication did not include taxa now commonly encountered in Great Lakes samples. For *Holopedium*, we assumed that development

Table 5. Bělehrádek's coefficients used to calculate egg development times (K) for zooplankton production estimates with the egg-ratio method, using the following equation $K = (A \times (T - \alpha)^{\beta})/24$. T is the mean temperature for the interval in ^{o}C. The calanoid coefficients were derived from the relationships in Cooley and Minns (1978). This table is from Johannsson et al. (2000).

Species	Bělehrádek's Coefficients		
	A	α	β
Bosmina/Eubosmina sp.	3750848	-15.40	-3.11
Daphnia sp.	65912	-6.10	-2.12
Diaphanosoma sp.	1767	-1.90	-1.08
Diacyclops/Tropocyclops	18901	-4.80	-1.77
Mesocyclops edax	19318	-0.48	-1.79
Cyclops vernalis	8128	-2.08	-1.67
Leptodiaptomus minutus	40994	-4.72	-2.00
L. ashlandi	40994	-4.72	-2.00
L.. sicilis	45284	-5.79	-2.00
L. siciloides	38148	-4.01	-2.00
Skistodiaptomus oregonensis	156046	-8.00	-2.31
Eurytemora affinis	38148	-4.01	-2.00
Epischura lacustris	38148	-4.01	-2.00

times were similar to those of *Daphnia.* Svensson and Gorokhova (2007) found that total egg development time of *Cercopagis* was 3.2 ± 0.43 days (mean ± SD) at 16^{o}C. Using Bělehrádek's coefficients, *Daphnia* development time at this temperature was only slightly longer (3.88 days). Therefore we used coefficients for *Daphnia* to estimate K for both *Holopedium* and *Cercopagis*. *Leptodora kindtii* development times in days were determined using the following equation where T is the average temperature in ^{o}C for the interval (Cummins et al. 1969):

$$K=-0.61*T+18.144 \qquad \text{[eqn 2]}$$

The development time equation for *Bythotrephes* is as follows:

$$K = (10\text{^}(6.840 - 9.305 \log(T) + 2.490 \log(T)^2)/12. \qquad \text{[eqn 3]}$$

This is based on Yurista's (1992) equation for determining development times from the start of the red-eye embryonic stage (mid) to the end of the black-eye embryonic stage (late). This is divided by 24 to convert from hours to days, then doubled to account for early embryonic development (prior to the red-eye stage). The resulting development times are also quite similar to *Daphnia*.

3.4. P/B PRODUCTION ESTIMATES

3.4.1. Overview

The production/biomass (P/B) method for calculating production can be used for taxa lacking reliable egg ratio estimates, including uncommon taxa for which only a few individuals were measured, as well as those that do not breed during the sampling period or drop their eggs after fertilization. As with ER production, it should also be calculated for individual thermal strata if possible. The model of Shuter and Ing (1997) predicts total zooplankton production over the growing season (gs) as follows:

$$P_{gs} = \text{daily P/B} \times B_{gs} \times D_{gs} \qquad \text{[eqn 4]}$$

where daily P/B is the average daily rate of production over the growing season, B_{gs} is the average population biomass over the growing season, and D_{gs} is the number of days in the growing season (e.g. 183 days for the May 1 to Oct. 31 period). Based on Johannsson et al. (2000), the daily P/B rate is determined with the following equation:

$$\log (\text{daily P/B}) = A + 0.04336 * T_{med} \qquad \text{[eqn 5]}$$

where T_{med} is the median water temperature for the sampling season in ^{o}C and A is -1.844 for cyclopoids, -2.294 for calanoids and -1.631 for rotifers. Stockwell and Johannsson (1997) give a value of -1.725 for cladocerans. For the larger, predatory cladocerans *Bythotrephes* and *Leptodora kindtii*, the size-dependent equations of Stockwell and Johannsson (1997) are used to estimate daily P/B, where wt is the mean individual dry weight of animals in μg:

$T_{med} > 10^{o}$C $\quad \log (\text{daily P/B}) = -0.23 * \log (wt) - 0.73 \qquad$ [eqn 6]

$T_{med} < 10^{o}$C $\quad \log (\text{daily P/B}) = -0.26 * \log (wt) - 1.36 \qquad$ [eqn 7]

P/B zooplankton production has also been calculated in Lake Erie (Johannsson et al. 2000), the Bay of Quinte (Johannsson and Bowen, 2012) and Hamilton Harbour (Bowen and Currie 2017) using fixed daily P/B rates of 0.162 for cladocerans and 0.10 for veligers (Johannsson et al. 2000). These values were used in warm environments where $T_{med} > 10^{o}$C. For systems where $T_{med} < 10^{o}$C, fixed daily P/B rates of 0.042 for cladocerans and 0.04 for veligers were used. These fixed daily P/B rates are not temperature dependent, and the warm rate represents a temperature of around 21.5^{o}C. The rationale for using this fixed rate is that epilimnetic water temperatures usually exceed 20^{o}C in these systems from June to September, the period when most of the annual cladoceran and veliger production occurs.

The warm veliger fixed daily P/B value was based on an increase in length from 60 to 250 μm over a 44 to 70 day period, representing an increase of 1.68 μg dry weight (Leach 1993, Doka 1994). This equates to a growth rate of 2.7 to 4.3 μm per day, and a daily P/B rate of 0.101 to 0.160 per day. Johannsson et al. (2000) used a daily P/B of

0.1 per day to be conservative and to allow for slower growth during the colder portions of the season. However, the settling period of up to 70 days given here is very long and the subsequent growth rates are low compared to other studies. Veliger settlement times of 18 to 28 days at temperatures of 16 to 24^{o}C are more commonly reported (e.g., Sprung 1989, Neumann et al. 1993, Wright et al. 1996), although Martel et al. (1993) estimated a planktonic period of 5 to 62 days in west-central Lake Erie. Sprung (1989) and Neumann et al. (1993) found length increases of 6 to 7.7 μm per day under optimal temperature and food conditions. Smylie (1995) reported growth rates of 3 to 20 μm per day in western Lake Erie, with a mean of 11.3 μm per day. Based on these results, it appears that the daily P/B estimate of 0.10 per day may be too low for warm epilimnetic samples, especially considering that the majority of veliger biomass now occurs in the summer and early fall when surface water usually exceeds 20^{o}C (Bowen et al. in press). Hillbricht-Ilkowska and Stanczykowska (1969) used a much higher veliger daily P/B rate of 0.24 to 0.27, which assumed veligers are in the plankton for only 8 days. Lazareva et al. (2016) used a daily P/B coefficient of 0.26, based on Alimov (1981). Based on these higher daily P/B rates, veliger production in Lake Erie and the Bay of Quinte would be about 2.5 times higher than the values given in Johannsson et al. (2000) and (Johannsson and Bowen, 2012). However, the 0.10 per day value in Johannsson et al. (2000) may still be reasonable for less productive, open-lake systems where the epilimnion tends to be cooler, such as offshore Lake Ontario (e.g., median temperatures of 15 to 18^{o}C). The daily veliger P/B rate of 0.04 per day for systems where T_{med} <10^{o}C may be adequate for the hypolimnion where *D. bugensis* often resides. For the metalimnion where temperatures may be in the 11 to 13^{o}C range, an intermediate daily P/B rate of 0.06 may be more realistic.

It also should be noted that the settling periods and growth rates given here are largely based on zebra mussel (*Dreissena polymorpha*) veligers and not quagga mussels (*D. bugensis*), the species that has largely replaced *D. polymorpha* in the Great Lakes (Wilson et al. 2006). There is very little information available on growth rates for *D. bugensis* veligers and more research needs to be done to determine these values over a range of temperatures and food concentrations typically found in the Great Lakes to accurately predict veliger production.

3.4.2. Improving P/B Production Estimates

Moving forward, a better approach that works in all temperate systems is to use temperature dependent daily P/B rates rather than fixed rates, and incorporate a taxon-specific median temperature that reflects the period when the majority of animals from that taxon are present. For example, there may be few *Daphnia* present in May, so May temperatures should be excluded when determining the median temperature used in calculating the daily P/B rate for *Daphnia*. P/B estimates may be further improved by calculating production for each time interval during the sampling season, which is similar to the method used to determine ER production. The mean temperature and biomass for each time interval would be used in the calculations, and the production estimates for each interval are added up to provide an estimate of total production (P_{int}) across the sampling season.

We recalculated production using the interval method (P_{int}) for cyclopoids and calanoids (pooling taxa of each group together) and compared to the original method (P_{GS}). This was done for warm, relatively shallow systems including the Bay of Quinte, Hamilton Harbour, western Lake Erie and the reference sites (mostly nearshore Lake Ontario), as well as cooler, less productive sites in central-east Lake Erie and the offshore station LO81 in Lake Ontario (Table 7; Appendix 3). To see how P_{int} and P_{GS} production differed for cladocerans, we used *Bosmina* at LO81 and the Bay of Quinte as examples. Despite the idea that production based on the interval method should yield more accurate results, production values based on the two methods were usually quite similar. Agreement was closest in warm, shallow systems, where the median temperatures across the sampling season did not radically deviate from conditions found during times of peak biomass of various groups. Most groups in these systems had mean P_{int}: P_{GS} ratios close to 1 in Table 7. However, if the P_{int} values tended to be higher than the P_{GS}, then the majority of biomass for that group occurred at temperatures higher than the median. This was observed for diaptomid calanoids at most stations, for cyclopoids and the calanoid group *Limnocalanus* + *Epischura* in the deeper stratum of LO81 and *Bosmina* in the epilimnion of LO81. The reverse was true for cyclopoids in Hamilton Harbour and *Bosmina* in the cooler lower Bay of Quinte. When regression analyses were performed on ln-transformed values of P_{int} and P_{GS}, the relationships were almost all significant ($p<0.05$), and r^2 values were usually greater than 0.80 (Table 7). The epilimnetic cyclopoid relationship at LO81 was the only one that was not significant, probably due to high annual variability.

3.5. COMPARISON OF EGG RATIO AND P/B PRODUCTION METHODS

3.5.1. Overview of Regression Analyses

For the locations listed in Section 3.4.2 and marked with a * in Appendix 3, ln-transformed annual P/B production estimates (P_{gs}) were regressed against ln-transformed ER estimates to determine agreement among the two methods (Table 6). These linear regressions were done for herbivorous cladocerans, *Bythotrephes*, *Cercopagis*, cyclopoids and diaptomid calanoids including *Eurytemora* (hereafter diaptomids), as well as some individual taxa. This was done to test the validity of using the original P/B technique to calculate production of the less common species or those with insufficient egg data. These linear regressions can also be used as correction factors to make P/B estimates more similar to those calculated using the ER method. Table 6 gives the slopes (a) and coefficients (b) for the correction equation:

$$\ln(ER_{pred}) = a \ln(P/B) + b \qquad \text{[eqn 8]}$$

where ER_{pred} is the predicted ER value and the P/B values were calculated using growing season means.

All linear regressions in Table 6 are statistically significant, and R^2 values for the significant relationships ranged between 0.49 and 0.95. The predicted ER values

Table 6. Linear regression results for production estimated by the P/B method vs. the ER method for various taxa at Great Lakes sites. ER production can be predicted using the relationship $\ln(ER_{pred}) = a*\ln(PB) + b$. For Strata, T=total, E=epilimnion, MH = metalimnion-hypolimnion. "Reference" represents sites in nearshore Lake Ontario, Lake Simcoe and Severn Sound, Georgian Bay.

Area Group	daily P/B*	stn	strata	Years	N	a	b	R^2	p	ER:P/B ratio ± SE
Bay of Quinte										
Herb. Cladoc.	F	B	T	95-02	18	1.023	-0.301	0.89	<0.001	1.32 ± 0.14
Herb. Cladoc.	F	HB	T	95-02	20	1.023	-0.480	0.83	<0.001	1.15 ± 0.12
Herb. Cladoc.	F	C	T	95-02	20	1.030	-0.955	0.80	<0.001	0.81 ± 0.09
Cyclopoids	TD	all	T	95-02	10	1.010	-0.696	0.93	<0.001	0.54 ± 0.03
Herb. Cladoc.	TD	B	T	95-08	57	1.125	-1.079	0.74	<0.001	1.10 ± 0.09
Herb. Cladoc.	TD	HB	T	95-08	69	0.938	0.508	0.61	<0.001	1.30 ± 0.13
Herb. Cladoc.	TD	C	T	95-08	65	0.968	0.279	0.64	<0.001	1.27 ± 0.11
Calanoids	TD	all	T	95-16	51	0.785	1.859	0.49	<0.001	2.01 ± 0.25
Hamilton Harbour										
Herb. Cladoc.	F	all	T	02 -12	78	0.968	-0.376	0.94	<0.001	0.57 ± 0.03
Herb. Cladoc.	TD	all	T	02 -12	80	1.029	-0.558	0.93	<0.001	0.81 ± 0.04
Cercopagis	TD	all	T	02 -12	16	1.091	0.147	0.93	<0.001	1.10 ± 0.11
Cyclopoids	TD	all	T	02 -12	19	1.292	-3.874	0.67	<0.001	0.43 ± 0.06
Calanoids	TD	all	T	02 -16	25	1.043	0.893	0.82	<0.001	3.61 ± 0.30
Lake Ontario										
Herb. Cladoc.	TD	LO81	E	07 -15	51	1.109	-0.834	0.90	<0.001	0.87 ± 0.06
Herb. Cladoc.	TD	LO81	MH	07 -15	42	1.129	-0.644	0.82	<0.001	1.03 ± 0.08
Herb. Cladoc.	TD	LO81	T	07 -15	93	1.084	-0.597	0.88	<0.001	0.94 ± 0.05
Cercopagis	TD	LO81	E	07 -15	9	1.243	-0.577	0.90	<0.001	1.71 ± 0.22
Cercopagis	TD	LO81	MH	07 -15	9	0.905	1.178	0.90	<0.001	0.63 ± 0.10
Cercopagis	TD	LO81	T	07 -15	18	0.978	0.883	0.95	<0.001	1.17 ± 0.18
Bythotrephes	WD	LO81	E	07 -15	9	1.045	0.981	0.82	0.001	3.30 ± 0.30
Bythotrephes	WD	LO81	MH	07 -15	8	0.917	0.586	0.92	<0.001	1.58 ± 0.27
Bythotrephes	WD	LO81	T	07 -15	17	1.139	0.282	0.92	<0.001	2.49 ± 0.29
Diacyclops[a]	TD	LO81	E	07 -15	7	1.397	-3.316	0.75	0.013	0.41 ± 0.09
Diacyclops[b]	TD	LO81	MH	07 -15	8	0.703	1.533	0.70	0.01	0.79 ± 0.12
L. sicilis	TD	LO81	E	07 -15	9	0.995	-0.633	0.73	0.003	0.63 ± 0.13
L. sicilis	TD	LO81	MH	07 -15	8	0.911	0.342	0.53	0.041	1.22 ± 0.24
Diaptomids	TD	LO81	MH	08 -16	8	1.017	1.173	0.67	0.007	3.99 ± 0.82
Herb. Cladoc.	TD	LO81	E	81 - 95	42	0.843	0.374	0.86	<0.001	0.64 ± 0.03
Diacyclops	TD	LO81	E	81 - 92	12	1.363	-1.097	0.85	<0.001	2.80 ± 0.31
Lake Erie										
Herb. Cladoc.	TD	C & E	T	93 - 98	48	0.951	-0.076	0.82	<0.001	0.90 ± 0.09
Herb. Cladoc.	TD	C & E	E	93 - 98	17	1.021	-0.663	0.89	<0.001	0.68 ± 0.08
Herb. Cladoc.	TD	west	T	93 - 98	43	1.047	-1.002	0.74	<0.001	0.76 ± 0.09
Cyclopoids	TD	all	T	93 - 98	27	0.956	-0.142	0.65	<0.001	0.81 ± 0.12
Diaptomids	TD	all	T	93 - 98	28	1.605	-3.431	0.79	<0.001	1.83 ± 0.19
Reference										
Herb. Cladoc.	TD	Ref.	T	09-16	60	0.949	-0.078	0.83	<0.001	0.79 ± 0.06
Cercopagis	TD	Ref.	T	09-16	13	1.471	-1.331	0.86	<0.001	2.18 ± 0.36
Bythotrephes	WD	Ref.	T	09-16	11	1.025	0.319	0.62	0.004	2.09 ± 0.49
Cyclopoids	TD	Ref.	T	09-16	25	0.841	0.508	0.76	<0.001	0.77 ± 0.10
Diaptomids	TD	Ref.	T	09-16	18	0.992	0.272	0.77	<0.001	1.70 ± 0.32

* TD = temperature-dependent daily p/B value; F= fixed value of of 0.162; WD =weight dependent relationship

[a] for P/B production less than 150 mg m^{-2}, the relationship $Er_{pred} = 0.505(P/B)$ was used.

[b] for P/B production less than 150 mg m^{-2}, the relationship $Er_{pred} = 0.772(P/B)$ was used.

Table 7. Linear regression results for ln-transformed P/B production estimated by the interval method (P_{int}) vs. temperature-dependent P/B production based on growing season means (P_{GS}) for various taxonomic groups. Interval production can be predicted using the relationship $\ln(P_{int})=a*\ln(P_{GS}) + b$. For "Strata", T =total, E=epilimnion and MH = metalimnion-hypolimnion. All stations are located in the Laurentian Great Lakes, with "Reference" sites in nearshore Lake Ontario, Lake Simcoe and Severn Sound, Georgian Bay. P/B production ratios of P_{int}: P_{GS} (± SE) are also given. The shaded p value is not significant.

Area	Taxa	Stn	Stratum	N	a	b	R^2	p	P_{int}:P_{GS}
Lake Erie									
	Diaptomids + *Eurytemora*	all	T	28	0.887	0.523	0.93	<0.001	1.16 ± 0.06
	Limnocalanus + *Epischura*	all	T	28	1.106	-0.309	0.94	<0.001	0.98 ± 0.05
	cyclopoids	all	T	28	0.858	0.746	0.90	<0.001	1.01 ± 0.07
Reference									
	Diaptomids + *Eurytemora*	all	T	29	0.967	0.198	0.97	<0.001	1.14 ± 0.05
	Limnocalanus + *Epischura*	all	T	20	0.904	0.205	0.92	<0.001	1.05 ± 0.07
	Cyclopoids	all	T	29	0.938	0.207	0.96	<0.001	1.00 ± 0.06
Hamilton Harbour									
	Diaptomids + *Eurytemora*	all	T	25	1.011	0.006	1.00	<0.001	1.06 ± 0.01
	Cyclopoids	all	T	25	0.779	1.321	0.82	<0.001	0.79 ± 0.03
Bay of Quinte									
	Diaptomids + *Eurytemora*	all	T	61	1.031	0.026	0.96	<0.001	1.13 ± 0.02
	Cyclopoids	all	T	69	1.058	-0.259	0.95	<0.001	1.07 ± 0.02
	Bosmina	B	T	22	1.16	-1.081	0.94	<0.001	0.95 ± 0.04
	Bosmina	HB	T	21	0.971	0.13	0.92	<0.001	0.96 ± 0.04
	Bosmina	C	T	19	1.055	-0.397	0.94	<0.001	0.85 ± 0.05
Lake Ontario									
	Diaptomids + *Eurytemora*	LO81	E	10	0.470	1.566	0.50	0.023	1.23 ± 0.14
	Limnocalanus + *Epischura*	LO81	E	10	0.980	-0.031	0.80	<0.001	0.99 ± 0.09
	Diaptomids + *Eurytemora*	LO81	MH	10	1.159	-0.002	0.85	<0.001	1.46 ± 0.16
	Limnocalanus + *Epischura*	LO81	MH	10	1.262	-0.028	0.87	<0.001	1.29 ± 0.14
	Cyclopoids	LO81	E	9	0.315	1.951	0.21	0.22	1.01 ± 0.21
	Cyclopoids	LO81	MH	9	0.604	1.51	0.62	0.01	1.18 ± 0.20
	Bosmina	LO81	E	10	1.168	-0.089	0.88	<0.001	1.44 ± 0.06
	Bosmina	LO81	MH	10	1.060	-0.110	0.80	<0.001	1.05 ± 0.09

obtained from the equations in Table 6 are plotted against P/B production values for the various groups and locations in Figure 4. Scatter plots of measured ER and temperature-dependent P/B production values and associated trend lines are also plotted for herbivorous cladocerans (Figure 5), predatory cladocerans (Figure 6) and copepods (Figure 7) at various Great Lakes locations. In general, the extent to which the temperature-dependent P/B method overestimates or underestimates production compared to the ER method depends on both the taxonomic group and location, as shown by the mean ER:P/B ratios in Table 6 and the trend lines in Figure 4.

3.5.2. Cladocerans

The two methods usually gave similar results for herbivorous cladocerans, although the ER method on average yielded slightly higher production values in the Bay of Quinte, with ratios ranging between 1.10 ± 0.09 at Belleville and 1.30 ± 0.13 at Hay Bay. In contrast, total water column ER estimates were slightly lower than P/B estimates at the remaining sites, with ratios ranging from 0.76 ± 0.09 in the west basin of Lake Erie to 0.94 ± 0.05 at LO81. Ratios were similar between the epilimnion (epi) and metalimnion-hypolimnion (MH) at LO81, and between deep (stratified) and shallow (unstratified) sites in Hamilton Harbour (Figure 5). The 2014 Lake Erie herbivorous cladoceran relationship is also very similar to the relationship observed during the 1990s in the central and eastern basins (Figure 5F), suggesting these earlier equations still apply to Lake Erie. The ER to P/B production trend lines for bosminids and *Daphnia* were almost identical in Lake Erie (Figure 8A). However, in the epi of LO81, ER production tended to be highest for bosminids, intermediate for *Daphnia* and lowest for *Holopedium* for a given P/B value (Figure 8B).

The two methods yielded similar production values for *Cercopagis* in Hamilton Harbour. For LO81 in Lake Ontario, the P/B method overestimated production in the MH (0.63 ± 0.10) but underestimated production in the epi (1.71 ± 0.22). The P/B method also underestimated *Bythotrephes* production this site, with ratios of 3.30 ± 0.30 in the epi and 1.58 ± 0.27 in the MH. For the reference stations, the P/B method yielded lower values for both *Cercopagis* (ratio = 2.18 ± 0.36) and *Bythotrephes* (2.09 ± 0.49). The ln-transformed relationships for *Cercopagis* in Hamilton Harbour and the reference sites, and for *Bythotrephes* in Lake Erie in 2014 and the reference sites were all quite similar to the relationships seen in the epi of LO81 (Figure 6). It is noteworthy that *Bythotrephes* P/B production is weight-dependent (Stockwell and Johannsson 1997), and not temperature-dependent. This may explain why P/B production values tend to deviate farther from the ER estimates for this species, especially at cooler open-lake sites.

3.5.3. Cyclopoids

In contrast, the P/B method has tended to overestimate cyclopoid production in recent years. When cyclopoid species were pooled, ER:P/B ratios were 0.81 ± 0.12 in Lake Erie, 0.54 ± 0.03 in the Bay of Quinte, 0.43 ± 0.06 in Hamilton Harbour and 0.77 ± 0.10

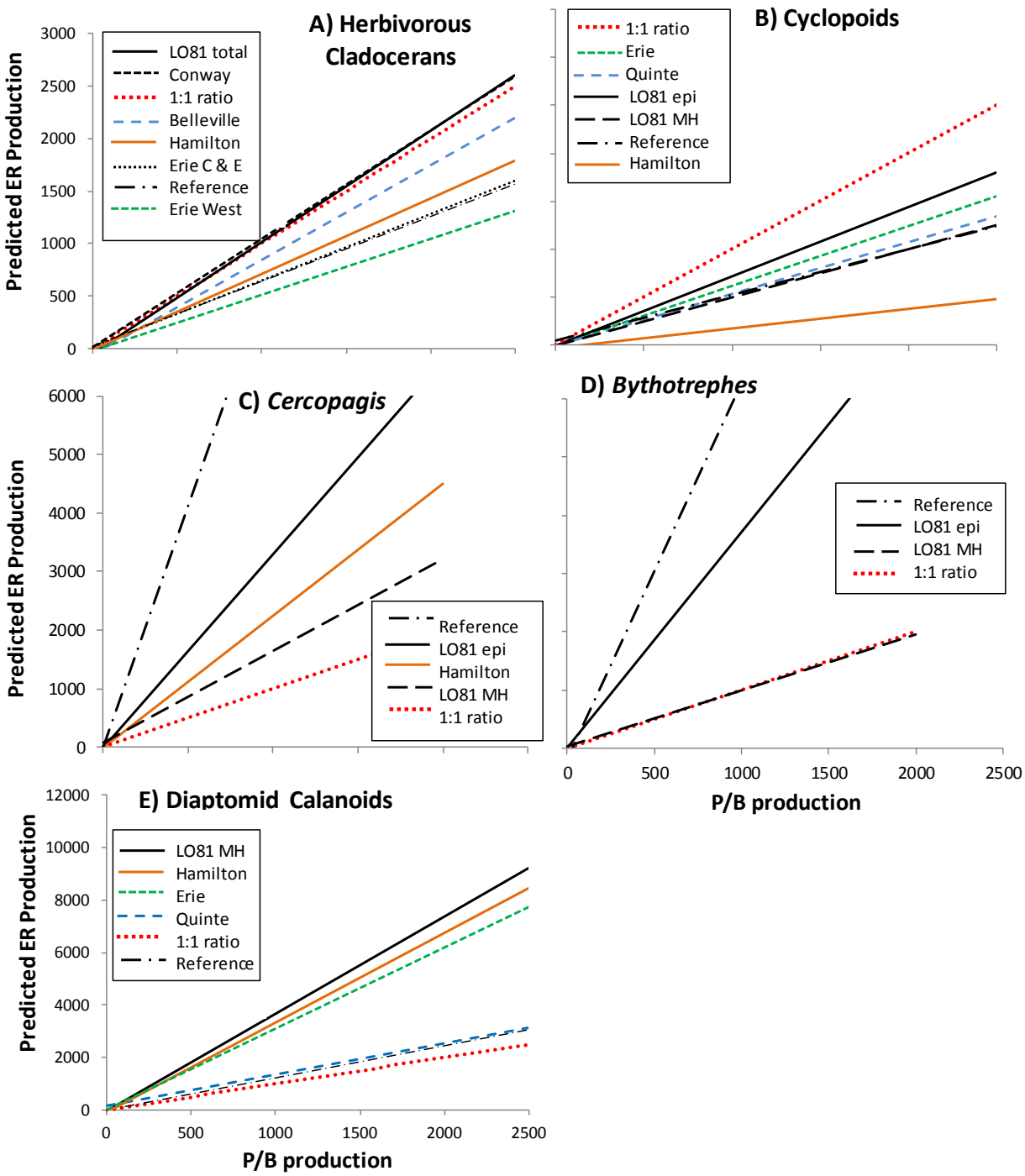


Figure 4. Predicted ER Production based on the relationship $\ln(ER_{pred})=a*\ln(PB)$ as given in Table 6 for A) herbivorous cladocerans, B) cyclopoids, C) *Cercopagis,* D) *Bythotrephes*, and E) Diaptomid calanoids at various Great Lakes Locations. LO81 is in the Kingston Basin of Lake Ontario. Reference sites are located in nearshore Lake Ontario, Lake Simcoe and Severn Sound in Georgian Bay.

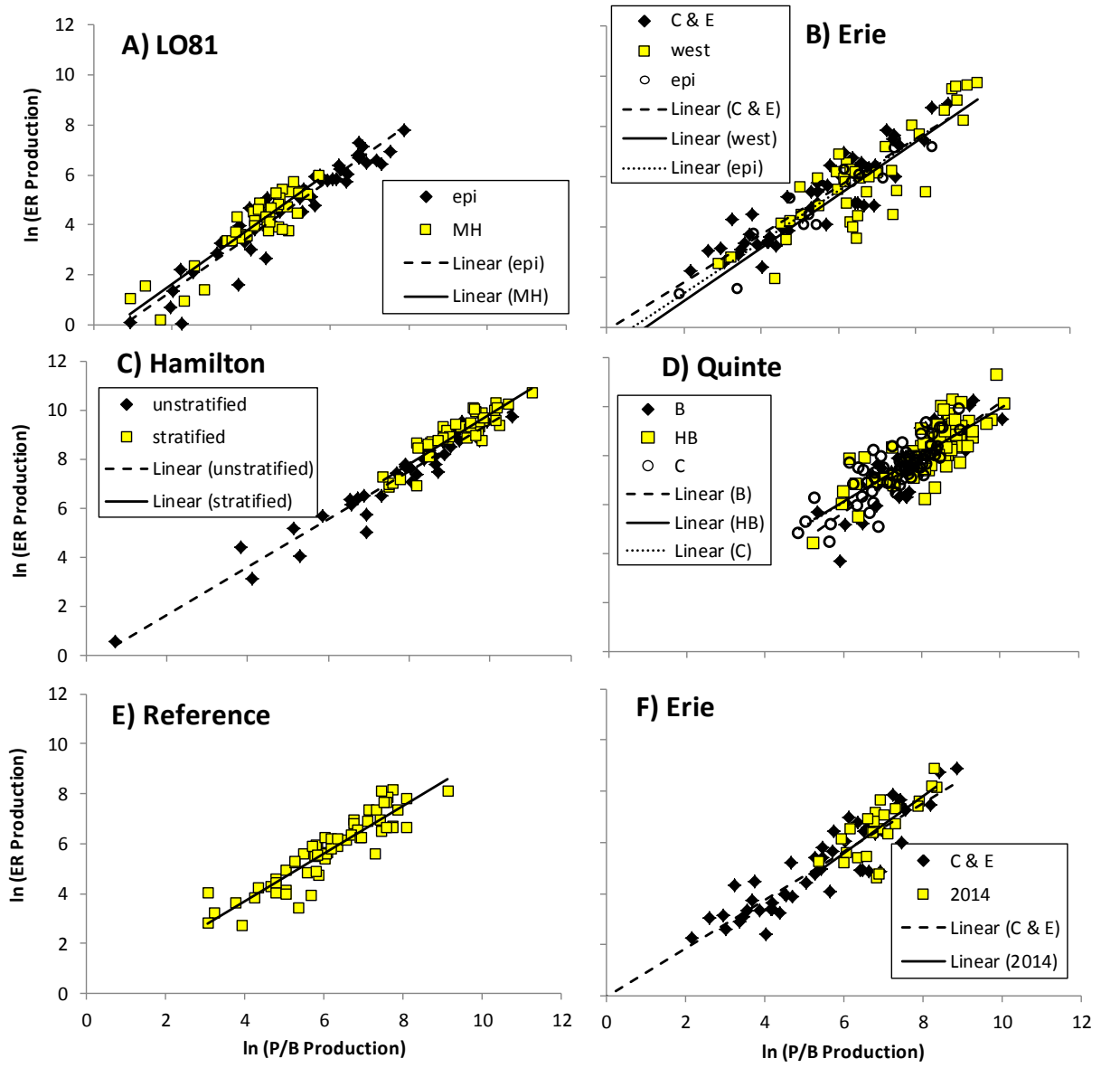


Figure 5. Relationships between total May to October production of herbivorous cladocerans determined using the ER method and the temperature-dependent P/B method. Production is given as natural log-transformed areal production (mg m^{-2}). A) LO81 in Lake Ontario includes the epilimnion (epi) and the meta-hypolimnion (MH). B) Lake Erie includes the western basin and the central and eastern basins (C & E). C) Hamilton Harbour samples are from shallow unstratified sites and deeper stratified sites. D) Bay of Quinte stations are Belleville (B), Hay Bay (HB) and Conway (C). E) Reference nearshore stations in western and central Lake Ontario, Lake Simcoe and Severn Sound. F) 1990s central and east (C & E) sites in Lake Erie are compared to lake-wide Erie sites in 2014.

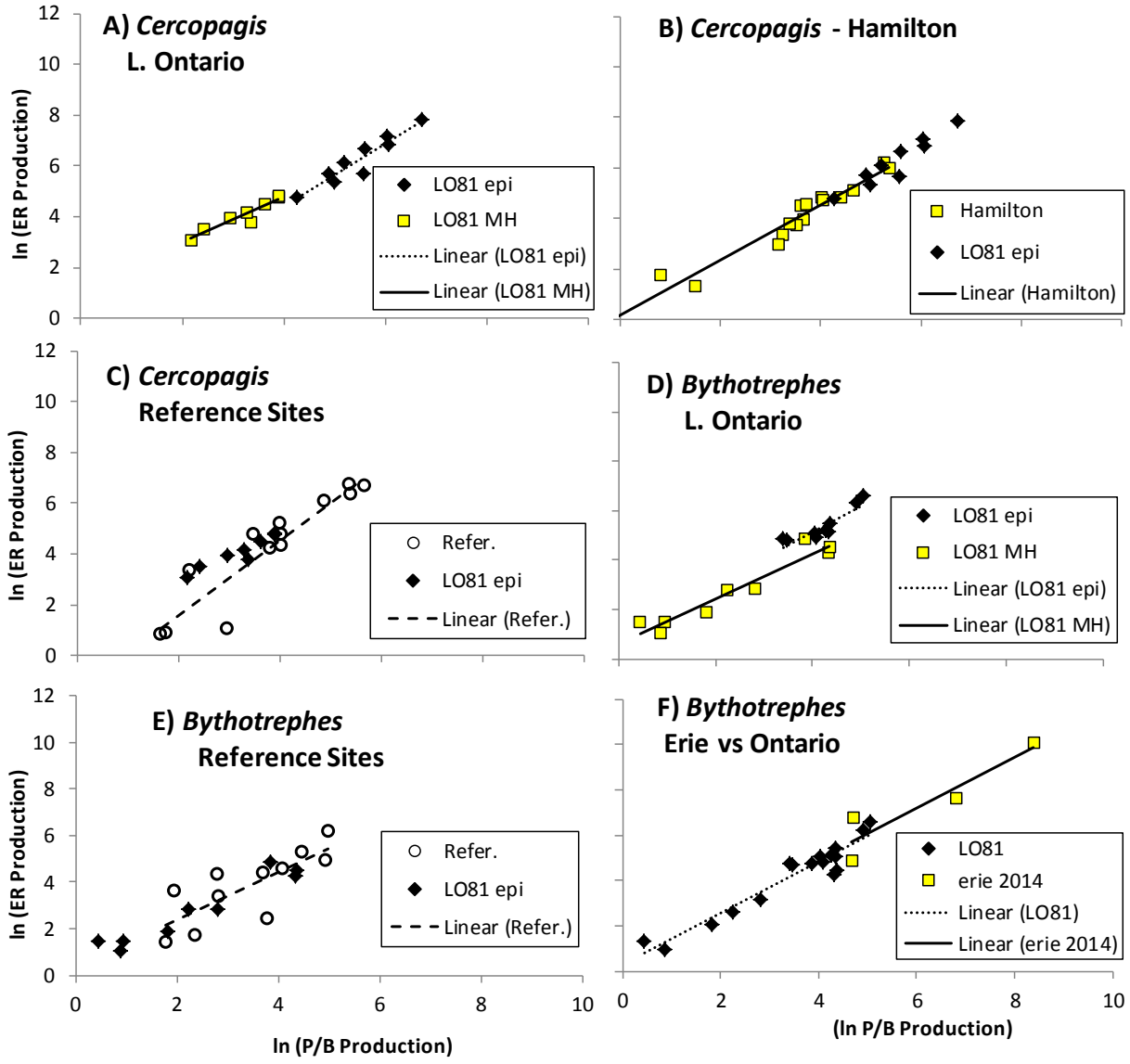


Figure 6. Relationships between total May to October production of predatory cladocerans determined using the ER method and the P/B method. Production is given as natural log-transformed areal production (mg m^{-2}). A) *Cercopagis* in Lake Ontario, including the epilimnion (LO81 epi) and the meta-hypolimnion (LO81 MH) from 2007 to 2015. B) *Cercopagis* in Hamilton Harbour (all sites combined). C) *Cercopagis* at "Reference" sites in the nearshore of Lake Ontario. D) *Bythotrephes* from LO81 in Lake Ontario. E) *Bythotrephes* from nearshore reference sites in Ontario, Lake Simcoe and Georgian Bay. F) *Bythotrephes* at Lake Erie sites sampled in 2014. Values at each site are compared to LO81 epi.

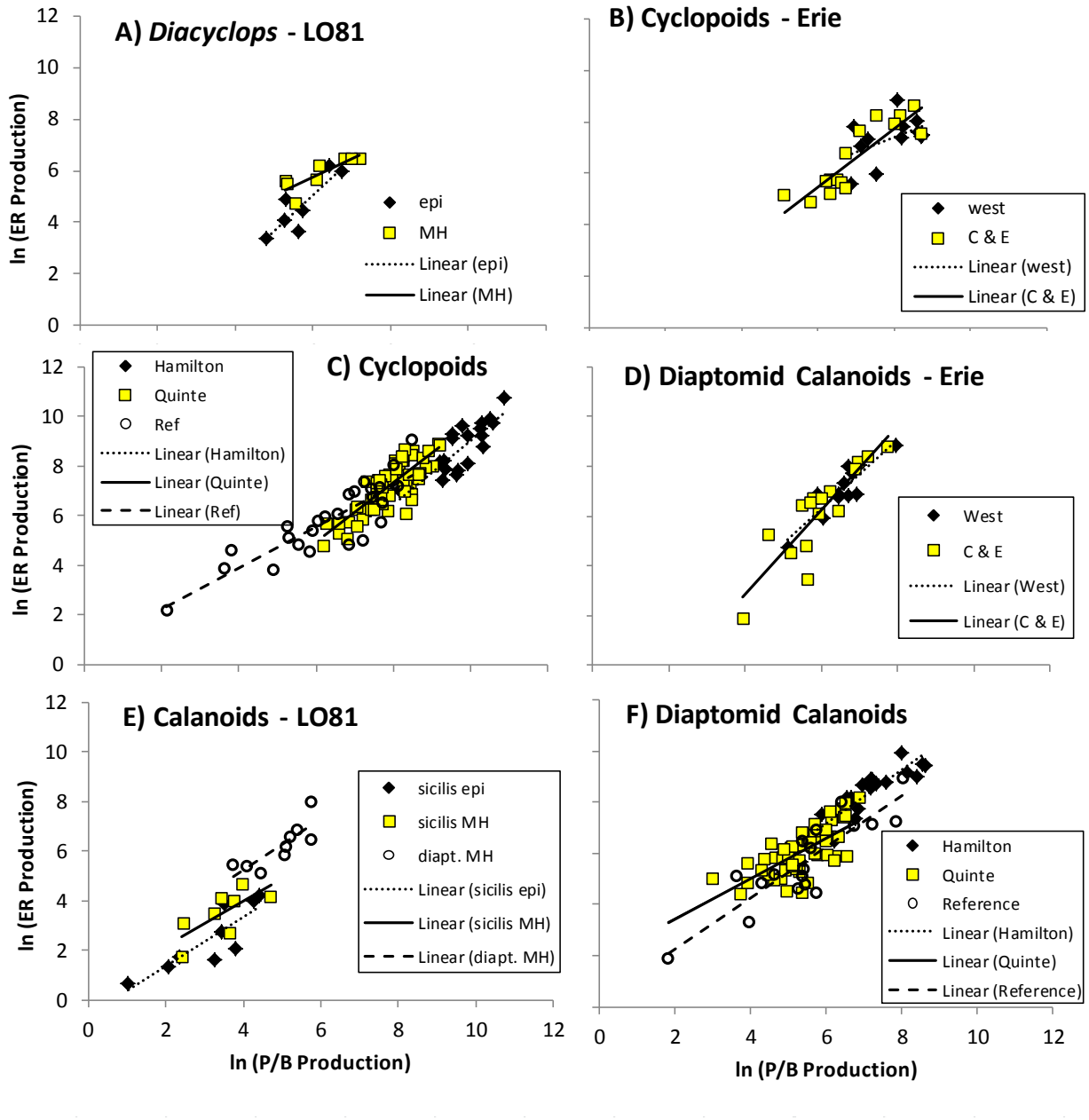


Figure 7. Relationships between total May to October production of copepods determined using the egg ratio (ER) method and the variable temperature P/B method. Production is given as natural log-transformed areal production (mg m^{-2}). A) Cyclopoid *Diacylops thomasi* at LO81 in Lake Ontario. B) Cyclopoids (taxa pooled) in Lake Erie from the western and the central and eastern basins (C & E) in the 1990s. C) Cyclopoids from the Bay of Quinte, Hamilton Harbour and reference sites. D) Diaptomid calanoids and *Eurytemora* (taxa pooled) in Lake Erie. E) Calanoid *Leptodiaptomus sicilis* from the epi and meta-hypo of LO81, compared to diaptomid calanoids from the meta-hypo. F) Diaptomids from Quinte, Hamilton Harbour and reference sites.

in the reference areas. In Lake Erie, the cyclopoid relationships were similar in the western basin and in the central and eastern basins and therefore all stations were pooled together. For *Diacyclops thomasi*, the dominant cyclopoid in Lake Ontario, P/B estimates were also usually higher than ER values at LO81, with mean ratios of 0.41 ± 0.09 in the epi and 0.79 ± 0.12in the MH. However, the opposite was true for epilimnetic *Diacyclops* during the 1981 to 1992 period at LO81, when the ER estimates were higher (Figure 9B). By 1993, ER production for this species had dropped to the range seen in recent years. For the early period at LO81, the ER to P/B relationship was not significant for *Tropocyclops*, the second most abundant cyclopoid. There were not enough gravid individuals of this genus to estimate ER for the later period. There were also some differences between *Diacyclops* and *Tropocyclops* in Lake Erie in the 1990s (Figure 8C).

3.5.4. Calanoids

The relationships between ER and P/B production tends to be more variable for calanoids and may depend on both taxon and location (Figure 7C and D; Figure 8D). Johannsson and Bowen (2012) and Bowen and Currie (2017) used unadjusted P/B estimates for calanoids in the Bay of Quinte and Hamilton Harbour, respectively, as they felt there were insufficient data to develop correction equations.

We recalculated calanoid ER production for each year and station in these systems by pooling together biomass and egg densities of all diaptomids and *Eurytemora* taxa, rather than calculating each taxon separately. The calanoid communities in Quinte and Hamilton Harbour are dominated (>90%) by these taxa. Diaptomid ER production was also determined using this method for sites in Lake Erie, the reference stations and Lake Ontario's LO81. Within each system, all stations were pooled as the ER:P/B relationships among sites were consistent. Based on these new calculations, the P/B method substantially underestimates diaptomid production at most sites (Table 6). ER production values in the MH at LO81 were on average four times higher than P/B values, although the epilimnetic regression was not significant due to high annual variability. The ER:P/B ratios averaged between 1.7 and 3.6 at the remaining sites.

When calanoid taxa were examined separately at LO81, the ER:P/B ratio was 0.63 ± 0.13 in the epi and 1.22 ± 0.24 in the MH for *Leptodiaptomus sicilis*. No significant relationship was found for *S. oregonensis*, and there were not enough eggs found for ER estimates for the other taxa. The calanoids *Limnocalanus* and *Epishura* were excluded from these analyses as ER production could not be calculated for these species. Given the absence of egg data, it is unknown how accurate the available P/B estimates are for these species and no correction factors are available at this time.

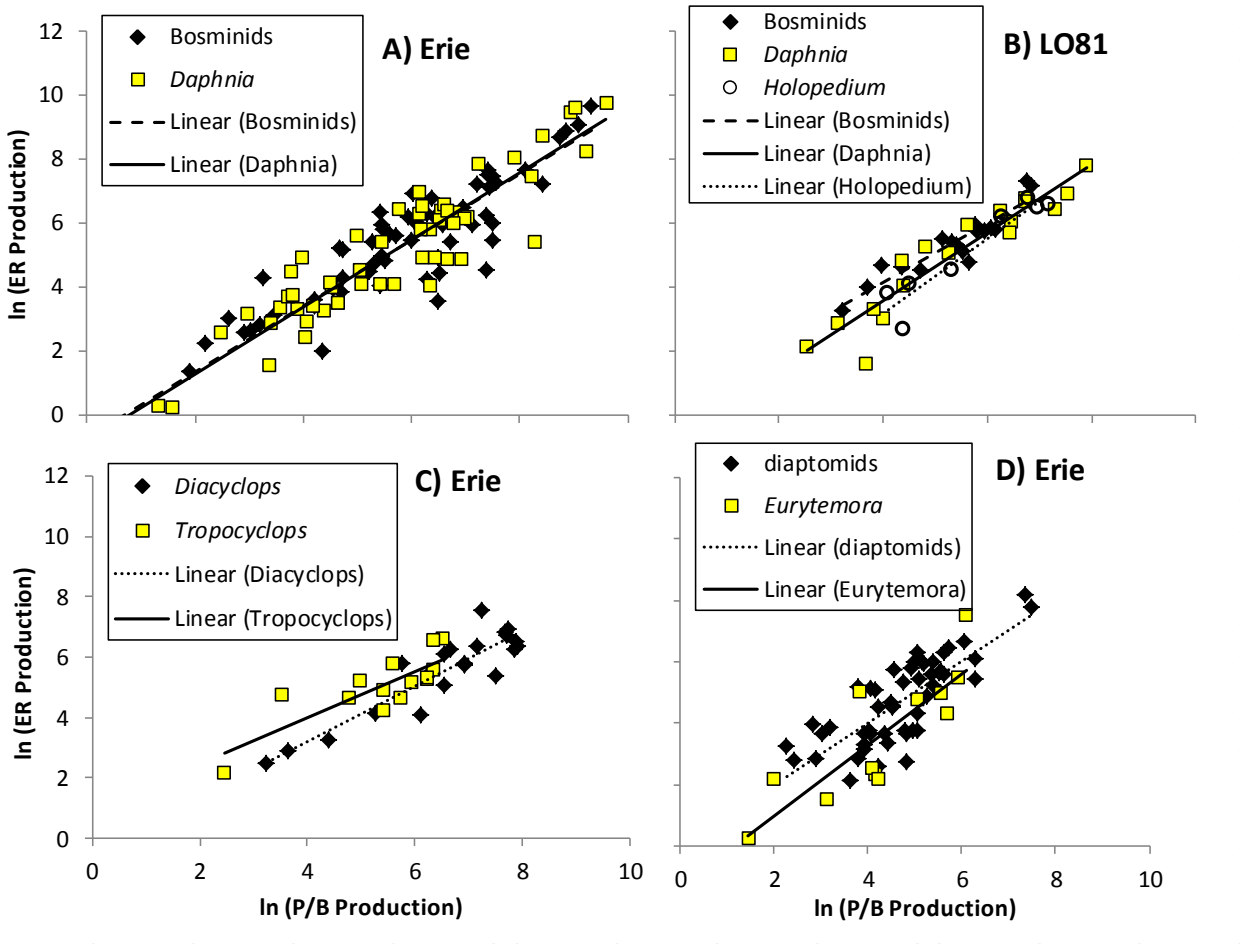


Figure 8. Relationships between total May to October production of various taxa determined using the ER method and the variable temperature P/B method. Production is given as natural log-transformed areal production (mg m^{-2}). A) Herbivorous cladoceran taxa in Lake Erie in 1993, 1994 and 1998, combining all basins. B) Cladoceran taxa in the epilimnion of LO81 in Lake Ontario from 2007 to 2015. C) Cyclopoid taxa and D) Calanoid taxa in Lake Erie from 1993, 1994 and 1998, combining all basins.

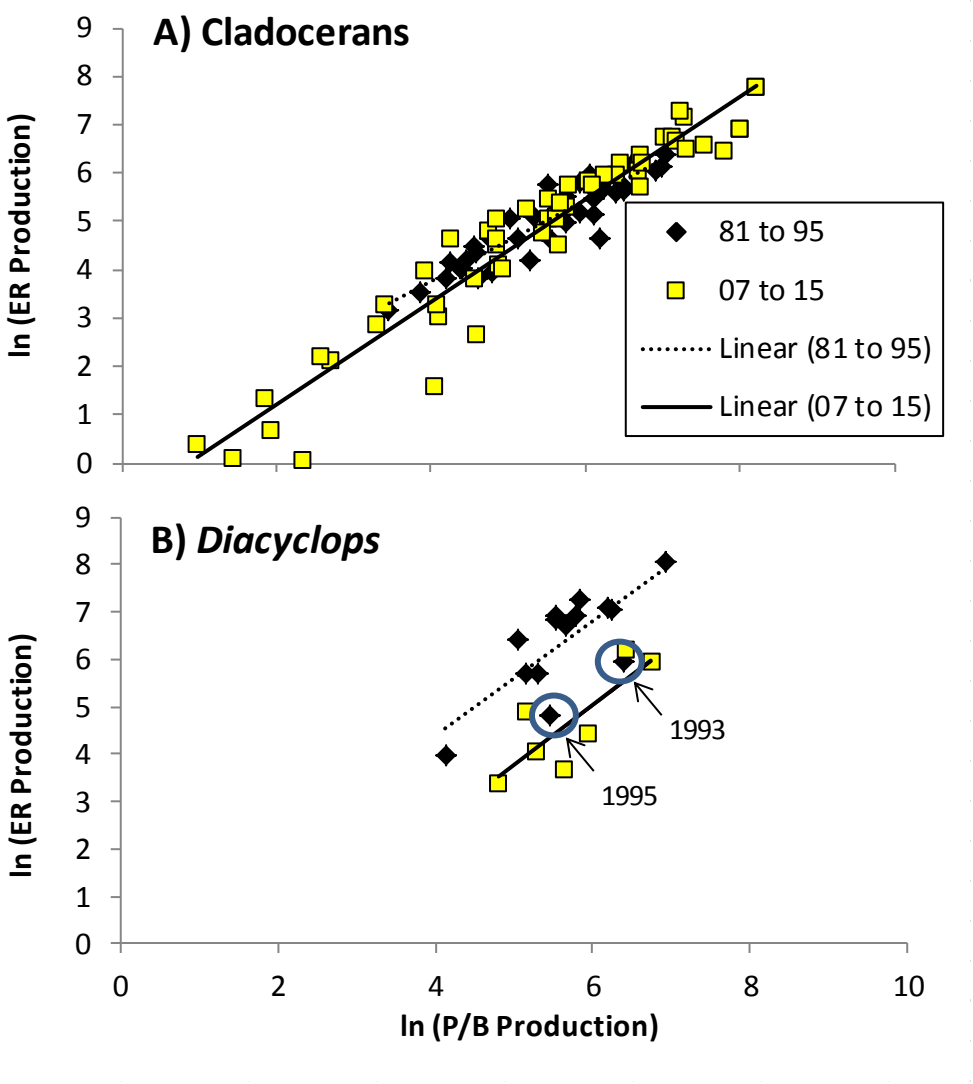


Figure 9. Relationships between total May to October production of zooplankton determined using the egg ratio (ER) method and the variable temperature P/B method. Epilimnetic production is given as natural log-transformed areal production (mg m^{-2}) at LO81 in Lake Ontario from two time periods: 1981 to 1995 and 2007 to 2015. Groups are A) herbivorous cladocerans and B) cyclopoid *Diacylops thomasi*. Note that ER production data were not available for 1994, and that *Diacyclops* production for the last two years of the early time stanza (1993 and 1995) were more similar to the recent time stanza.

3.5.5. Application of P/B Correction Factors

The application of herbivorous cladoceran and cyclopoid correction equations to predict ER production for less abundant taxa has been done for the Bay of Quinte (Johannsson and Bowen 2012) and Hamilton Harbour (Bowen and Currie, 2017). Regression coefficients differed among Quinte stations for cladocerans, but not copepods. Relationships among stations in Hamilton did not differ. Given that these are both warm embayments, these publications used a fixed daily P/B value of 0.162 for herbivorous cladocerans, based on a temperature of 21.5^{o}C (Table 6). We compared results obtained using this fixed value to temperature-dependent daily P/B rates (Figure 10). Production estimates were very similar for herbivorous cladocerans at Belleville in the Bay of Quinte because this is a shallow unstratified station where median seasonal temperatures are usually very close to 21^{o}C. Hay Bay and the Hamilton Harbour stations are usually deeper and slightly cooler, so the temperature-dependent P/B estimates were slightly lower than the "fixed" values. The largest difference was seen at Conway, a 33 m deep stratified site in the lower Bay of Quinte where the median water temperature is usually around 15^{o}C (range of 12.9 to 18.6^{o}C).

For Lake Erie data from the 1990s, Johannsson et al. (2000) multiplied P/B production estimates by the following correction factors: 3 for diaptomid and *Eurytemora* calanoids, 0.71 for cyclopoids, and 0.75 for the cladocerans *Bosmina* and *Eubosmina*. Other cladocerans and calanoids were not corrected. These factors were simply the mean ER:P/B ratios for each group. These ratios used in Johannsson et al. (2000) were generally similar to the recalculated values for each group in Table 6. However, our work shows the relationships for *Daphnia* and bosminids to be very similar (Figure 8A). As Johannsson et al. (2000) only corrected P/B production of bosminids, unadjusted values for *Daphnia* and other herbivorous cladocerans may have been overestimated in Lake Erie.

In summary, it is hoped that the Great Lakes equations presented in Table 6 will help to guide zooplankton researchers performing secondary production calculations in a variety of freshwater temperate systems. Using the ER method, which incorporates actual egg data and biomass for each time interval, is the preferred method of determining zooplankton production. However, when egg data are insufficient, the accuracy of P/B production estimates can be improved by applying the appropriate correction factors.

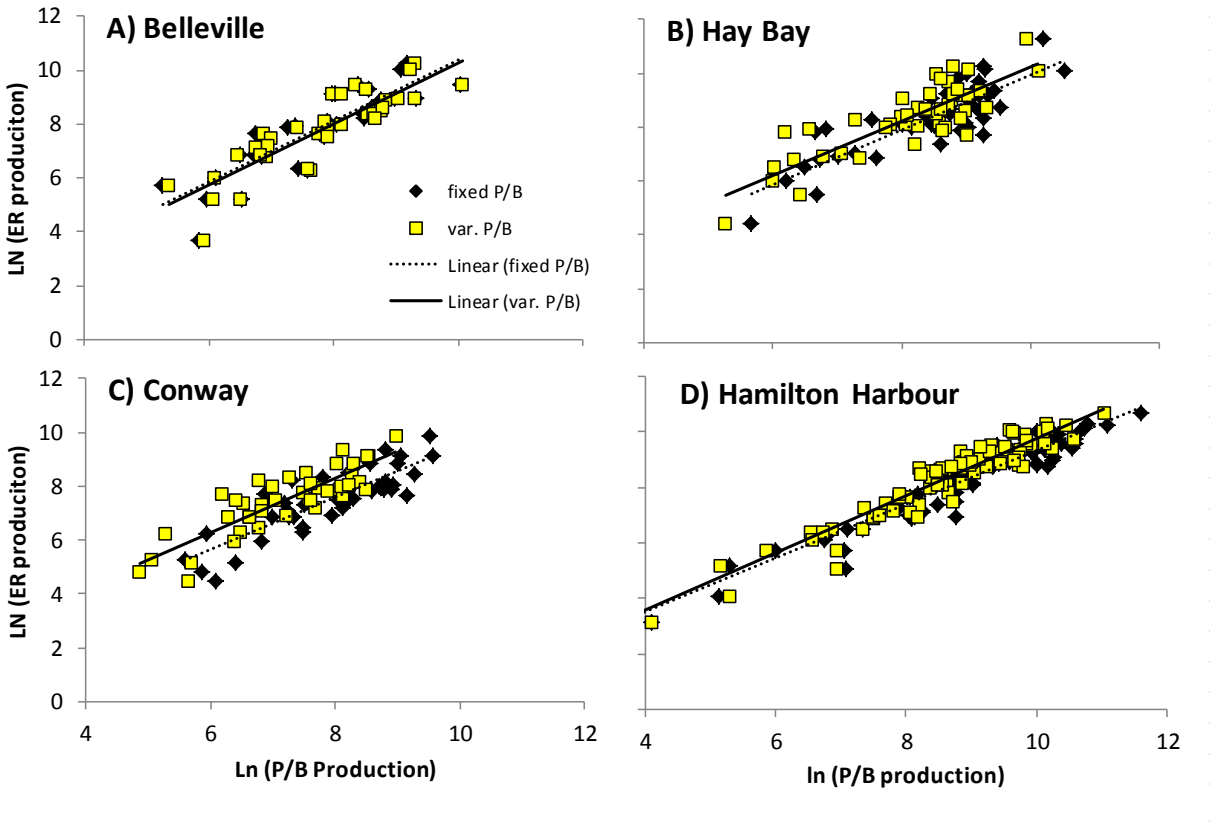


Figure 10. Relationships between total May to October production of herbivorous cladocerans determined using the egg ratio (ER) method and the P/B method. Production is given as natural log- transformed areal production (mg m^{-2}). Relationships are shown for A) Belleville, B) Hay Bay and C) Conway in the Bay of Quinte and for D) Hamilton Harbour. The fixed (warm) P/B values were calculated using a daily PB value of 0.162, and the var. P/B values were calculated using variable, temperature-dependent daily P/B values.

4.0 ACKNOWLEDGEMENTS

Foremost, I acknowledge my indebtedness to Dr. O. Johannsson who, with the help of S. Millard, M. Munawar and other research scientists at Fisheries and Oceans Canada in Burlington, ON, established most of the sampling programs that provided data for this report. Dr. Johannsson, with the help of Dr. J. Cooley, J. Moore and others, developed the laboratory counting protocols and zooplankton production methods outlined here. I am grateful for the insightful reviews and comments provided by A. Conway, J. Watkins and R. Rozon. Thanks to W.J.S. Currie and M. Koops for their technical advice and support in developing this document. I also wish to thank the field and laboratory crews who have made this work possible: J. Gerlofsma, R. Bonnell, A. Bedford, M. Frank, H. Niblock, M. Fitzpatrick, R. Rozon, T. Hollister, K. Bonnell, and others too countless to name. Thanks to C. Tudorancea, D. Geiling and W. Geiling for zooplankton sample enumeration and taxonomy. Financial support was provided by the Great Lakes Action Plan and by Fisheries and Oceans Canada.

5.0. REFERENCES

Alimov, A.F. 1981. *Funktsional'naya ekologiya presnovodnykh dvustvorchatykh mollyuskov* (Functional Ecology of Freshwater Bivalves). Leningrad: Nauka, Leningr. Otd.

Almond, M.J., Bentzen, R.E. and Taylor, W.D. 1996. Size structure and species composition of plankton communities in deep Ontario lakes with and without *Mysis relicta* and planktivorous fish. Can. J. Fish. Aquat. Sci. 53: 315–325.

Bottrell, H.H., Duncan, A., Gliwicz, Z.M., Grygierek, E., Herzig, A., Hillbricht-Ilkowska, A., Kurasawa, H., Larsson, P. and Weglenska, T. 1976. A review of some problems in zooplankton production studies. Norw. J. Zool. 24: 419-456.

Bowen, K.L., Conway, A.J. and Currie, W.J.S. In press. Could dreissenid veligers be the lost biomass of invaded lakes? Freshwater Sci.

Bowen, K.L. and Currie, W.J.S. 2017. Elevated zooplankton production in a eutrophic Lake Ontario embayment: Hamilton Harbour 2002–2014. Aquat. Ecosyst. Health 20: 230-241.

Bowen, K.L. and Johannsson, O.E. 2011. Changes in zooplankton biomass in the Bay of Quinte with the arrival of the mussels, *Dreissena polymorpha* and *D. rostiformis bugensis*, and the predatory cladoceran, *Cercopagis pengoi*: 1975 to 2008. Aquat. Ecosyst. Health 14: 44-55.

Chick, J.H., Levchuk, A.P, Medley, K.A., and Havel, J.H. 2010. Underestimation of rotifer abundance a much greater problem than previously appreciated. Limnol. Oceanogr.: Methods 8: 79–87.

Cooley, J.M. and Minns, C.K. 1978. Prediction of egg development times of freshwater copepods. J. Fish. Res. Board Can. 35: 1322-1329.

Cooley, J.M., Moore, J.E., and Geiling, W.T. 1986. Population dynamics, biomass and production of the macrozooplankton in the Bay of Quinte during changes in phosphorus loadings. *In* Project Quinte: point-source phosphorus control and ecosystem response in the Bay of Quinte, Lake Ontario, *Edited by* C.K. Minns, D.A. Hurley, and K.H. Nichols. Can. Spec. Publ. Fish. Aquat. Sci. 86: 166–176.

Culver, D.A., Boucherle, M.M., Bean, D.J. and Fletcher, J.W. 1985. Biomass of freshwater crustacean zooplankton from length-weight regressions. Can. J. Fish. Aquat. Sci. 42: 1380-1390.

Cummins, K.W., Costa, R.R., Rowe, R.E., Moshiri, G.A., Scanlon R.M. and Zajdel, R.K. 1969. Ecological Energetics of a Natural Population of the Predaceous Zooplankter *Leptodora kindtii* Focke (Cladocera). Oikos 20: 189-223.

Doka, S.E. 1994. Spatio-temporal Dynamics and Environmental Prediction of Recruitment in *Dreissena* Populations of Lake Erie. M.Sc. Thesis. University of Guelph, Guelph, Ont. pp.138.

Downing, J. A. and Rigler, F. H. 1984. A manual on the methods for the assessment of secondary productivity in lakes. 2nd ed. Blackwell Scientific Publications, Oxford.

Dumont, H.J., Van de Velds, I. and Dumont, S. 1975. The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton and benthos of continental waters. Oecolog. 19: 75-97.

Grigorovich, I.A., MacIsaac, H.J., Rivier, I.K., Aladin, N.V. and Panov, V.E. 2000. Comparative biology of the predatory cladoceran *Cercopagis pengoi* from Lake Ontario, Baltic Sea and the Caspian Lake. Arch. Hydrobiol. 149: 23–50.

Hillbricht-Ilkowska, A. and Stanczykowska. A. 1969. The production and standing crop of planktonic larvae of *Dreissena polymorpha* (pall.) in two Mazurian lakes. Polskie Archiwum Hydrobiologii 16:193-203.

Humphreys, W.F. 1981. Towards a simple index based on liveweight and biomass to predict assimilation in animal populations. J. Anim. Ecol. 50: 543–561.

Huntley, M.E. and Lopez, M.D.G. 1992. Temperature-dependent production of marine copepods: a global synthesis. Am. Nat. 140: 201–242.

Johannsson, O.E. 1995. The response of *Mysis relicta* population dynamics and productivity to spatial and temporal gradients in Lake Ontario. Can. J. Fish. Aquat. Sci. 52: 1509-1522.

Johannsson, O.E, Bowen, K. L. 2012. Zooplankton production in the Bay of Quinte 1975–2008: relationships with primary production, habitat, planktivory, and aquatic invasive species (*Dreissena* spp. and *Cercopagis* pengoi). Can. J. Fish. Aquat. Sci. 69, 2046–2063.

Johannsson, O.E., Dermott, R., Graham, D.M., Dahl, J.A., Millard, E.S., Myles, D.D. and LeBlanc, J. 2000. Benthic and pelagic secondary production in Lake Erie after the invasion of *Dreissena* spp. with implications for fish production. J. Great Lakes Res. 26: 31–54.

Kerfoot, W.C., Budd, J.W., Green, S.A., Cotner, J.B., Biddanda, B.A., Schwab, D.J. and Vanderploeg, H.A. 2008. Doughnut in the desert: Late-winter production pulse in southern Lake Michigan. Limnology and Oceanography. 53: 589-604.

Lazareva, V.I., Kopylov, A. I., Sokolova, E. A. and Pryanichnikova, E. G. 2016. Veliger larvae of dreissenids (Bivalvia, Dreissenidae) in the plankton foodweb of Rybinsk Reservoir. Biology Bulletin 43:1313–1321.

Leach, J.H. 1993. Impacts of the zebra mussel, (*Dreissena polymorpha*) on water quality and spawning reefs in western Lake Erie. pp. 381-397. In Nalepa T. and Scholesser, D. (eds). Zebra Mussels: biology, impacts and control. Lewis Publishers, Boca Raton, Florida.

Lee, W. Y. and McAlice, B. J. 1979. Sampling variability of marine zooplankton in a tidal estuary. Estuar. & Coast. Mar. Sci., 8: 565-82.

Mack, H.R., Conroy, J. D., Blocksom, K.A., Stein, R.A. and Ludsin, S.A. 2012. A comparative analysis of zooplankton field collection and sample enumeration methods. Limnol. Oceanogr.: Methods 10: 41–53.

Malley, D.F., Lawrence, S.G., MacIver, M.A. and Findlay, W.J. 1989. Range of variation in estimates of dry weight for planktonic Crustacea and Rotifera from temperate North American lakes. Can. Tech. Rep. Fish. Aqua. Sci. No. 1666: 49 pp.

Martel, A. 1993. Dispersal and recruitment of zebra mussel (*Dreissena polymorpha*) in a nearshore area in west-central Lake Erie: The significance of postmetamorphic drifting. Can. J. Fish. Aquat. Sci. 50: 3-12.

Marty, J., Bowen, K. and Koops, M.A. 2011. Erratum to: Distribution and ecology of *Hemimysis anomala*, the latest invader of the Great Lakes basin. Erratum to: Hydrobiologia (2010) 647: 71–80.

McCauley, E., 1984. The estimation of the abundance and biomass of zooplankton in samples. pp. 228–265. In Downing, J.A. and Rigler, F. H. (eds). A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters, Second Edition. Blackwell Scientific Publications, Oxford.

McEwen, G.F., Johnson, M.W. and Folsom, T.R. 1954. A statistical analysis of the performance of the Folsom plankton sample splitter, based upon test observations. Arch. Met. Geophys. Bioklim., Ser. A, 7: 502-27.

Neumann, D., Borcherding, J. and Jantz,. B. 1993. Growth and seasonal reproduction of *Dreissena polymorpha* in the Rhine River and adjacent waters. pp. 95-109. In Nalepa, T. and Scholesser, D. (eds). Zebra Mussels: biology, impacts and control. Lewis Publishers, Boca Raton, Florida.

Paloheimo, J.E. 1974. Calculations of instantaneous birth rate. Limnol. Oceanogr. 19: 692-694.

Plante, C. and Downing, J.A. 1989. Production of freshwater invertebrate populations in lakes. Can. J. Fish. Aquat. Sci. 46: 1489– 1498.

Rosen, R.A. 1981. Length-dry weight relationships of some freshwater zooplankton. J. Freshwat. Ecol. 21: 225-229.

Selgeby, J.H. 1975. Life histories and abundance of crustacean zooplankton at the outlet of Lake Superior, 1971-1972. J. Fish. Res. Board. Can. 32: 461-470.

Sell, D.W. and Evans, M.S. 1982. A statistical analysis of subsampling and an evaluation of the Folsom plankton splitter. Hydrobiologia 94: 223-230.

Shuter, B.J. and Ing, K.K. 1997. Factors affecting the production of zooplankton in lakes. Can. J. Fish. Aquat. Sci. 54: 359–377.

Smylie, P.H.P. 1995. Growth and abundance of Dreissena polymorpha larvae in relation to temperature and phytoplankton abundance. MSc thesis, University of Guelph.

Sprung, M. 1989. Field and laboratory observations of *Dreissena polymorpha* larvae: abundance, growth, mortality and food demands. Arch Hydrobiol 115: 537-561.

Stockwell, J., and Johannsson, O.E. 1997. A temperature-dependent, allometric model to estimate zooplankton production in temperate, freshwater lakes. Can. J. Fish. Aquat. Sci. 54: 2350–2360.

Strecker, A.L. and Arnott, S.E. 2008. Invasive predator, *Bythotrephes*, has varied effects on ecosystem function in freshwater lakes. Ecosystems 11: 490–503.

Svensson, S. and Gorokhova, E. 2007. Embryonic development time of parthenogenically reproducing *Cercopagis pengoi* (Cladocera, Onychopoda) in the northern Baltic proper. Archiv für Hydrobiologie 170: 257–261.

Vanderploeg, H.A., Bolsenga, S.J., Fahnenstiel, G.L. Liebig, J.R. and Gardner, W.S. 1992. Plankton ecology in an ice-covered bay of Lake Michigan: utilization of a winter phytoplankton bloom by reproducing copepods. Hydrobiologia 243: 175-183.

Van Guelpen, L., Markle, D.F. and Duggan, D.J. 1982. An evaluation of accuracy, precision, and speed of several zooplankton subsampling techniques. J. Cons. int. Explor. Mer. 40: 226-236.

Warner, D., Rudstam, L.G., Benoît, H., Mills, E.L. and Johannsson, O. 2006. Changes in seasonal nearshore zooplankton abundance patterns in Lake Ontario following establishment of the exotic predator *Cercopagis pengoi.* J. Great Lakes Res. 32: 531–542.

Wilson, K.A., Howell, E.T. and Jackson, D.A. 2006. Replacement of zebra mussels by quagga mussels in the Canadian nearshore of Lake Ontario: the importance of substrate, round goby abundance, and upwelling frequency. J. Great Lakes Res. 32: 11-28.

Wright, D.A., Setzler-Hamilton, E.M., Magee, J.A., Kennedy, V.S. and McIninch, S.P. 1996. Effect of salinity and temperature on survival and development of young zebra (*Dreissena polymorpha*) and quagga (*D. bugensis*) mussels. Estuaries 19: 619-628

Yurista, P.M. 1992. Embryonic and post-embryonic development in *Bythotrephes cederstroemii*. Can. J. Fish. Aquat. Sci. 49: 1118-1125.

Appendix 1. Zooplankton Enumeration Methods used by DFO's GLLFAS Lab in Burlington, Ontario

Equipment

- Fume hood or similar well-ventilated area
- personal protective equipment (gloves, eye protection, lab coat)
- Beakers of various sizes (e.g. 250 mL, 500 mL)
- Graduated cylinder for beaker calibration
- Funnel with mouth larger than diameter of beakers
- 64µm sieve smaller than funnel mouth
- 400 µm sieve that nests into 64µm sieve
- Wash bottle with water
- Wash bottle with 4% sugar buffered formalin
- Formalex® Green – formalin neutralizing agent
- Labelled waste formalin container (e.g. 4 L glass bottle or plastic jug)
- Hensen Stempel pipette (1 mL or 2 mL)
- Petri dishes – square grid-marked dishes work well for whole sample counts
- Zooplankton counting chamber – e.g. 6 mL Bogorov chamber, Ward counting wheel
- Dissecting microscope (e.g. 10 to 40x or greater magnification) mounted with digital camera
- Software to conduct measurements on digital images
- 6 or 8-channel tally counters; one-channel tally counters
- Bench sheets or notebook for recording data

Methods

1. Begin by setting up a station in the fume hood or other well ventilated area to remove preservative from the zooplankton sample. Use proper personal protective equipment for formalin use (gloves, eye protection, lab coat).

2. If whole-sample *Cercopagis* and/or *Bythotrephes* enumeration is necessary, nest a small 400 µm sieve inside a 64 µm sieve to separate the large and small animals. Otherwise just use a 64 µm sieve. Place the sieve(s) in a funnel over a beaker ("Waste Formalin") (Fig. A1). Pour the contents of the zooplankton sample into the nested sieves and wait until most of the formalin has drained away.

3. Transfer the funnel and sieve(s) over another beaker ("Rinse") and using a wash bottle with water, rinse any remaining material from the inside of the sample jar and lid into the sieve. Gently rinse any residual formalin from the sample using the wash bottle, allowing the spray to permeate the sample fully.

4. If *Cercopagis* or *Bythotrephes* enumeration is not required and only the 64 µm sieve was used, proceed to step 6. Otherwise, gently flush smaller animals through the 400 µm sieve and into the 64 µm sieve with water.

5. Wash the contents of the 400 µm sieve into a petri dish and enumerate *Cercopagis* and/or *Bythotrephes* in the entire sample. Square plastic petri dishes with grid lines make counting easier. If the sample is particularly dense, it may be necessary to divide it into several petri dishes. If present, measure and count eggs in up to 30 individuals of each taxon. Alternatively, set aside a minimum of 30 to 50 or more individuals of each taxon for future measurement. Spare animals are useful in case some are damaged. These can be stored in labelled 0.5 mL or 1.5 mL bullet tubes and preserved with 4% sugar buffered formalin. Return the remaining 400 µm fraction to the 64 µm sieve.

6. Place the funnel and 64 µm sieve with the rinsed sample over a third beaker, flip the sieve upside down into the funnel and using the wash bottle, rinse the entire zooplankton sample into the beaker. The size of the beaker used will depend on the density of zooplankton/material in the sample. Very dense samples, such as net hauls from productive systems should be placed in a 500 mL or larger beaker, whereas a smaller beaker is adequate for low volume samples from unproductive areas. Be sure that the beakers have been calibrated for the correct volumes. The volume markings on most standard laboratory beakers may not be accurate – adding a known volume of water from a graduated cylinder can confirm these markings.

7. Using water in a wash bottle, dilute the sample in the beaker to a known volume and record this as “Working Volume”. Again, this volume will depend on the concentration of material in the sample.

8. Using the Hensen Stempel (HS) pipette (Fig. 2), mix the zooplankton sample thoroughly in the beaker. In order to optimize randomization in the beaker, a figure eight mixing pattern is required. Mixing is incredibly important and potentially a large source of error. Heavier taxa such as veligers tend to settle to the bottom of the sample quickly. If the sample is not mixed properly and the pipette is close to the bottom, an over representation of veligers may occur. Some taxa also float on the surface of the water, which can influence counting. If animals are floating, add a drop of dishwashing detergent to break the surface tension.

9. To check whether the sample dilution is appropriate, take an aliquot of the sample using the HS pipette and place it in a petri dish. Rinse the HS pipette chamber into the dish with a small amount of water. Scan the sample under a dissecting microscope to check organism density, particularly for the abundant taxa. Make adjustments to the aliquot or working volume if it is too dense. Some trial and error may be required to determine the best volume for a given set of samples. Rinse the contents of all petri dishes scanned into the empty 64 µm

sieve. This may be placed in a petri dish containing water to prevent the organisms from drying out prior to re-preservation.

10. Take additional aliquot(s) of the sample using the HS pipette (step 8). Carefully empty each aliquot into the counting chamber, rinsing the pipette chamber with water after each addition (Fig. 3). Ward counting wheels or 6 mL Bogorov chambers may be used for enumeration. Fill the counting chamber with enough water to distribute the aliquot(s) throughout the chamber. Record the volume of aliquot(s) added - the number of aliquots used will depend on the sample density, but remember that the entire counting chamber must be enumerated. Animals do not randomly distribute themselves in the chamber so subsampling at this point is not possible. To count abundant small animals such as veligers or nauplii, it may be desirable to initially count only one aliquot. Alternatively, skip enumerating overly abundant animals in the first chamber, and count an additional chamber where fewer or more dilute aliquots were used (see “Troubleshooting” section).

11. Systematically examine the zooplankton counting chamber using 32x – 40x magnification and make notes of rotifers, algae blooms, sediment etc. Six to eight channel tally counters or a set of one channel tally counters are useful for enumeration. Count and record all zooplankton taxa to the desired taxonomic level as follows:

    - Loose cladoceran eggs (including neonates too small to be living independently) and copepod eggs
    - Adult cyclopoids to genus. Juvenile cyclopoids may simply be classified as cyclopoid copepodids.
    - Cladocerans to genus. If possible, distinguish *Daphnia retrocurva* from *Daphnia galeata mendotae*. Helmet shape may aid in this identification, along with the presence/absence of the ocellus on fresh samples. The presence of “teeth” on the postabdominal claw is a true identifier of *D. retrocurva*, but this may require slide mounting specimens and viewing under a higher power compound microscope. If necessary, use 85% lactic acid as a mounting medium for clearing specimens.
    - Veligers and copepod nauplii - distinguishing calanoid and cyclopoid nauplii is generally too difficult for most enumerators.
    - Calanoids (adults and later stage copepodids) should be split into the following groups: *Limnocalanus*, *Epischura*, diaptomids and *Eurytemora*. However, it may not be feasible to easily separate the latter two taxa, especially at the copepodid stage. Identification of diaptomid calanoids to species may also require slide mounting and is usually not undertaken due to the expertise and time required.

12. For biomass estimation, measurements of animal lengths are usually required. These may be obtained using a digital imaging system such as Northern Eclipse (Empix, Mississauga, ON, CA) or the public domain software ImageJ. Measuring points are given in Figure 2. Depending on their density, up to 30 to 50 animals in

each taxon should be measured. For production estimates, egg counts are also required. Count any attached eggs in the individuals measured above.

13. Once you have enumerated the entire counting chamber, rinse the contents into the 64 µm sieve. Do not return to the remaining sample in the beaker as this will result in additional dilution. Depending on animal density, count additional aliquots to ensure 200-400 animals have been counted from the sample. Once adequate numbers of a particular taxon have been counted in an aliquot (e.g. 50 to 100 individuals), it is not necessary to count that taxon in additional aliquots. However, more aliquots may be necessary to estimate densities of rarer taxa. For example, it may be necessary to count only 0.5% of the sample for nauplii and veligers, but 3 to 5% for larger, less abundant taxa. Record the aliquot volumes for each taxon. Remember that for any given aliquot, the entire counting chamber must be enumerated. Recording counts and aliquot volumes for each counting chamber will provide information on subsampling variability for that sample.

14. For less abundant taxa, it may be easier to perform animal measurements in a petri dish. When counts are complete, add a portion (or all) of the remaining sample into a petri dish. Start in one corner and measure the animals as you find them. This should ensure that animals are randomly chosen for measurement, not just the larger individuals that are easier to spot.

15. When the sample has been counted and measured, pour the remaining sample into the 64 µm sieve. You should now have the entire zooplankton sample back in the sieve. Drain out any water. Place the funnel and sieve over the original sample jar and carefully rinse the sample back into the jar using 4% sugar buffered formalin. If sample has not yet been archived, transfer it to a properly labelled flint archive bottle. When measurements of any *Cercopagis* or *Bythotrephes* (previously removed from the sample and stored in a bullet tube) have been completed, the bullet tube can be added to the archived sample.

16. When finished, a small amount of Formalex® Green can be used to neutralize any diluted formalin in the rinse water so that it can be poured down the drain safely. Undiluted non-neutralized formalin waste should be stored in a labeled container for proper disposal. Alternatively, clean undiluted formalin drained from the sample may be used to re-preserve the sample at the end of the counting process.

Troubleshooting

When counting zooplankton, one or several taxa may be high in abundance and not feasible to count. There are two options to solve this:

1. You can continue counting the remaining low density taxa in the counting chamber (200-400 animals should be counted for each zooplankton sample) and skip the highly abundant ones. On your next aliquot, remove a smaller volume

aliquot and count everything. Record the aliquot volume at which each taxon was counted. This method is useful for when one or two taxa are abundant.

2. If the entire sample is too abundant, you can adjust the working volume to dilute the density of all the animals. Be sure to record the changes in the working volume in your notebook.

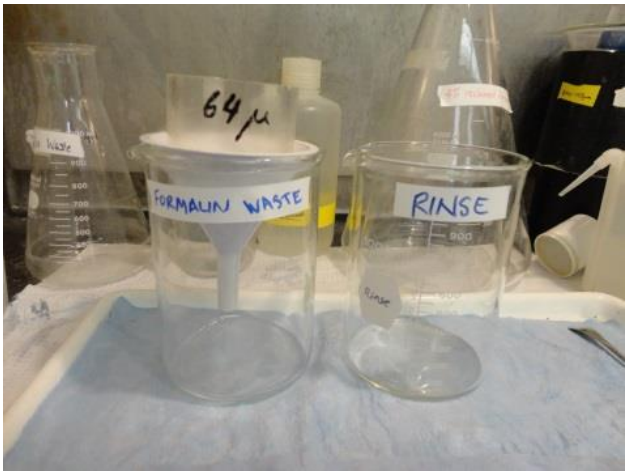
In both scenarios, aliquot volumes need to be diligently recorded for each taxon. Your note book or bench sheets should have the information given in the example below, plus any other information pertinent to that sample.

Example:

Site: A89	Date: Sept 8, 2014	Rep: 2	Strata: Total	Time: Day	Gear: 153 net	
Haul Volume: 809 L	Working Vol: 100 mL					NOTES
	Animal Counts					Many Asplanchna, some K. quadrata, Ceratium present
	2 mL Aliquot	1 mL Aliquot	Total Aliquot	Fraction Counted	Density (No.L^{-1})	
Diaptomid	100	45	145	0.03	5.97	
Bosmina	nc*	150	150	0.01	18.54	
Bythotrephes	nc*	nc*	25	1	0.03	

*nc - the aliquot was not counted for that taxon

In this example, *Bosmina* were very abundant in the first aliquot of 2 mL, so they were over looked and the remaining taxa were counted. In the second aliquot, 1 mL was taken and all taxa were counted. The entire sample was enumerated for *Cercopagis* or *Bythotrephes*, so the fraction counted is 1. The “fraction counted” column is “total aliquot” volume enumerated divided by the working volume. The density is the “total aliquot count” divided by the “fraction counted”, then divided by the haul volume.



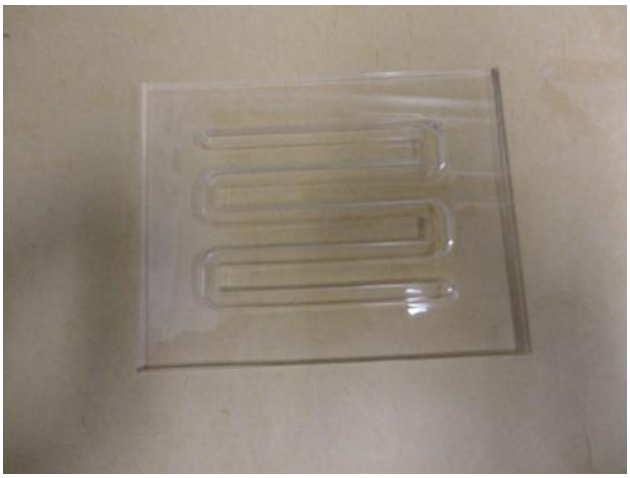


Figure A1. Beaker set up for rinsing in fume hood.
Figure A2. Hensen Stempel pipettes.
Figure A3. 6 mL Bogorov counting chamber.

Appendix 2: Literature sources used to identify zooplankton and rotifers

Balcer, M.D., Korda N. L. and Dodson, S. I. 1984. Zooplankton of the Great Lakes: A guide to the identification and ecology of the common crustacean species. The University of Wisconsin Press, Madison, Wisconsin.

Brandlova**,** J., Brandl, Z. and Fernando, C. H. 1972.The Cladocera of Ontario with remarks on some species and distribution. Can. J. Zool., 50: 1373-1403.

Brooks, J. L. 1957. The systematics of North American *Daphnia*. Mem. Conn. Acad. Arts & Sciences, 13: 1-180.

Dodson, S. I., and Frey, D. 1991. Cladocera and other Branchiopoda. Pp. 723-786 in Thorp, J.H., and Covich A. P.(eds.). Ecology and classification of North American freshwater invertebrates. Academic Press, San Diego.

Dumont, H. J. & Negrea S. V. 2002. Introduction to the Class Branchiopoda. Backhuys Publishers, Leiden, Belgium. 398 p.

Dussart, B. H. and Fernando, C. H. 1990. A review of the taxonomy of five Ontario genera of freshwater cyclopoid Copepoda (Crustacea). Can. J. Zool., 68: 2594-2604.

Dussart, B. H. and Fernando, C. H. 1990. Crustaces copepods de l'Ontario. University of Waterloo, Department of Biology.

Dussart, B. H. and Defaye, D. 2001. Introduction to the Copepoda. (2nd edition) (revised and enlarged). Backhuys Publishers, Leiden, Belgium. 344 p.

Dussart, B. H. and Defaye, D. 2002. World Directory of Crustacea Copepoda of Inland waters I – Calaniformes. Backhuys Publishers, Leiden, Belgium. 276 p.

Edmondson, W. T. (ed.). 1959. Freshwater Biology. Second Edition. John Wiley and Sons, Inc., New-York.

Korinek, V. 1981. *Diaphanosoma birgei* n.sp. (Crustacea, Cladocera). A new species from America and its widely distributed subspecies *Diaphanosoma birgei* ssp. *lacustris* n.ssp. Can. J. Zool., 59: 1115-1121.

Korovchinsky, N. M.1992. Sididae and Holopediidae (Crustacea: Daphniiformes). Backhuys Publishers, Leiden. 82 p.

Orlova –Bienkowskaja, M. Y. 2001. Cladocera: Anomopoda. Daphniidae: genus *Simocephalus*. Backhuys Publisher, Leiden, Belgium, 130.

Ruttner-Kolisko, A. 1974. Plankton Rotifers: biology and taxonomy. Die Binnengewasser 26 (1), 146 p.

Smirnov, N.N. 1996. Cladocera: the *Chydorinae* and *Sayciinae* (Chydoridae) of the World. SPB Academic Publishing, Amsterdam. 197 p.

Smith, K. and Fernando, C. H. 1978. A guide to the freshwater calanoid and cyclopoid copepod Crustacea of Ontario. University of Waterloo, Department of Biology, Ser. No.18.

Stemberger, R. S. 1979. A Guide to Rotifers of the Laurentian Great lakes. Environmental Monitoring and Support Laboratory**,** Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio. 45268. 185 p.

Wilson, M.S. 1959. Calanoida. Pp. 738-795 in Edmondson, W.T. (ed.) Freshwater Biology. Second Edition. John Wiley and Sons, Inc., New-York.

Appendix 3. Great Lakes Sampling Stations from 1981 to 2016.

* indicates station was used in the ER to P/B relationships; ** indicates it was one of the reference stations used in the relationships.

Area	Location	Station	Description	Depth (m)	Latitude	Longitude	Sampled years
Bay of Quinte		B*	Belleville	5	44.1537	-77.3456	1973-2016
		N	Napanee	5	44.1803	-77.0397	1973-1977, 1990-2009, 2011, 2014-16
		HB*	Hay Bay	11.5	44.0933	-77.0717	1973-2016
		P	Picton	10.7	44.0450	-77.1167	2010-11, 2014-2015
		GL	Glenora	17	44.0463	-77.0209	2010-11, 2013-2015
		C*	Conway	33	44.1089	-76.9089	1973-09, 2013-16
Lake Ontario							
	Eastern	LO81*	Kingston Basin	34	44.0167	-76.6717	1981-95, 2007-16
	Prince Edward County	WA**	Waupoos	6.7	43.9995	-76.9888	2009-2010
		WB**	Weller's Bay	6.3	43.9948	-77.5716	2012
		WL**	West Lake	5.2	43.9379	-77.2917	2012
	Central Basin	COB**	Cobourg	7.4	43.9504	-78.1649	1997, 2009-10
		LO41	centre	130	43.7167	-78.0267	1981-1997, 2003, 2008, 2013
	Western Basin	LO2**	Oakville offshore	60	43.3397	-79.6664	1995, 1999, 2013-2016
		BUR**	Burlington	7	43.2758	-80.0094	2013-2016
		BRO**	Bronte 5m	5	43.3781	-79.7097	2009-10, 2014
		BR15	Bronte 15m	15	43.3711	-79.7033	2009-10
		O2A	Bronte Pier	5	43.3929	-79.7059	2008-10
		PDAL**	Port Dalhousie	6	43.2189	-79.2512	2009
		O6A	Dalhousie pier	4	43.2097	-79.2632	2009
		LO8**	Humber Bay	12.5	43.6233	-79.4533	2003, 2008, 2013, 2016
		LO9	Toronto offshore	60	43.5867	-79.3950	2003, 2008, 2013
		LO12	Toronto offshore	106	43.5033	-79.3533	1981-84, 1990, 1996, 2007-09, 2012-14
		LO19	Niagara offshore	107	43.3833	-79.2850	2003, 2008, 2013

Appendix 3, cont'd.

Area	Location	Station	Description	Depth (m)	Latitude	Longitude	Sampled years
	Western Basin	LO18	Niagara offshore	88	43.3033	-79.2783	2003, 2008, 2013
		LO17	Dalhousie nearshore	14.6	43.2250	-79.2717	2003, 2008, 2013
		LO93	Niagara Bar	19.5	43.3267	-78.8683	1981-84
		LO207	Niagara Bar	72	43.3281	-79.0014	2014, 2015
Hamilton Harbour							
		HH258*	centre	23.5	43.2874	-79.8408	2002, 2007-10, 2012-16
		HH6*	LaSalle 6 m	6	43.3019	-79.8381	2002, 2007-09, 2012, 2014, 2016
		HH908*	west	14	43.2811	-79.8647	2003, 2008, 2013-14, 2016
		HH17*	Lasalle nearshore	1.5	43.3036	-79.8386	2002, 2008
		HHWC*	Willow Cove	3.5	43.2864	-79.8711	2003
		HH8	east	17	43.2944	-79.7997	2013-14, 2016
Lake Simcoe		S15**	offshore south	20.4	44.3426	-79.3903	2012
		C1**	Cook's Bay	3	44.2126	-79.5087	2012-2013
Severn Sound, Georgian Bay							
		P1**	Penetang dock	2.5	44.7722	-79.9460	2010, 2013
		P2	Penetang Bay	5.9	44.7855	-79.9384	2010
		P3	Penetang Bay	9.1	44.8059	-79.9403	2010, 2013
		P4**	Penetang Bay	11	44.8246	-79.9045	2010, 2013
		P5	Penetang Bay	16	44.8411	-79.8910	2010, 2013
		SSM1	Penetang Bay	7.8	44.8185	-79.9191	2010
Lake Erie							
	Eastern Basin	E1*		6	42.7795	-80.1450	1993-94, 1998
		E2*		37.6	42.6263	-80.0545	1993-94, 1998
		E3*		9	42.7137	-80.2295	1993-94, 1998
		E5*		2.6	42.6212	-80.3583	1998
		EC1*		12.3	42.5738	-80.6422	1998

Appendix 3, cont'd.

Area	Location	Station	Description	Depth (m)	Latitude	Longitude	Sampled years
	Eastern Basin	EC2*		21.5	42.4000	-80.6437	1998
		LE978*	east of Long Point tip	40	42.5501	-79.9903	2014
		LE977*	Long Point tip	6	42.5542	-80.0494	2014
		LE23*	east of Long Point tip	61.6	42.5333	-79.8990	1994
	Central Basin	C1*		9.1	42.5875	-81.4417	1998
		C2*		23.1	42.3587	-81.4428	1998
		WC1*		16.9	42.0767	-82.3400	1993, 1998
		WC2*		22.4	41.9833	-82.1400	1993
		LE84*	offshore	23.7	41.9361	-81.6631	1994
		LE946*	offshore w. of Erie PA	23	42.1667	-80.6416	1994
		LE979*	Point Pelee tip	4	41.9065	-82.5042	2014
		LE980*	east of Pelee tip	12	41.8853	-82.3823	2014
	Western Basin	W1*		10.1	41.9867	-82.5750	1993, 1998
		W2*		11.6	41.8833	-82.6133	1993, 1998
		W3*		10.9	41.8223	-82.9722	1993, 1998
		W6*		12.1	41.8542	-82.7633	1998
		W7*		10.1	41.9917	-82.7633	1998
		LE976*	Colchester Harbour	7	41.9795	-82.9362	2014
		LE975*	Central west basin	10.5	41.8566	-82.9856	2014
		LE970*	west	9.8	41.8250	-82.9750	1994
		LE358*	west	10.1	41.8942	-82.8683	1994

Appendix 4 – Calculating Zooplankton Production using the Egg Ratio Method

For each station for which production is to be calculated, there are n sampling dates for a given sampling season. Generally production is summed for the May 01 to Oct 31 period, but there are exceptions if the sampling season deviates markedly from this period. Production is calculated on an interval basis for each species, where there are n-1 intervals, each assigned the end date of the interval. The steps in this process are as follows:

1. Interval width (t) in days is computed for each of the n-1 intervals simply by subtracting the start date from the end date. If there are no Belehradek's coefficients (Table 5) for the current species, development times may be calculated using other means (e.g. *Leptodora, Bythotrephes, Cercopagis, Holopedium*).

2. A minimum density of 0.001 m^{-3} is added to each volumetric population density in the data array to prevent math errors in subsequent steps. If the user wants to calculate production results by area, all volumetric densities are then scaled by the current stratum thickness to get densities m^{-2}.

3. A development time in hours is computed using the Belehradek's coefficients:

   a) The mean (T) in degrees C of the temperatures at the interval start and end is computed.

   b) T is compared to Belehradek's alpha and if the T - a is greater than or equal to 1.01, the development time (d) in hours is computed as: d = A * (T - a) ^b

   c) Otherwise d is computed as: d = A.

   d) d is divided by 24 to get the rate in days.

4. A mean weight of adults plus juveniles is computed for each interval.

6. A mean weight of adults plus juveniles including eggs is likewise computed.

7. A mean egg-ratio is computed for each interval as ER and the corresponding instantaneous birth rate (b) for the interval is computed as: b=ln(1+ER)/d, where d is now in days.

8. The ratio of the total population density at the end of each interval to that at the start of the interval is obtained as R. The instantaneous population growth rate (r) for the interval is then computed as: r=ln(R)/t.

9. Production for each interval is calculated, completing the production table:

a) A mean weight (w) for the interval is used which is either the weight for adults plus juveniles or adults plus juveniles including eggs

b) If absolute value obtained for r is less than a minimum value of 0.005, a mean population density for the interval is computed as M and production (P) for the interval is computed as: P=M*b*w*t.

c) Otherwise, a difference (D) between the population densities at the end and start of the interval is computed and the production computed as: P=D*(b/r)*w.