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Plankton Trophic Structure Within Lake Michigan as Revealed By Stable Carbon and Nitrogen Isotopes

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PLANKTON TROPHIC STRUCTURE WITHIN LAKE MICHIGAN AS
REVEALED BY STABLE CARBON AND NITROGEN ISOTOPES

by

Zachery G. Driscoll

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ABSTRACT

PLANKTON TROPHIC STRUCTURE WITHIN LAKE MICHIGAN AS REVEALED BY STABLE CARBON AND NITROGEN ISOTOPES

by

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The University of Wisconsin-Milwaukee, 2014
Under the Supervision of Professor Harvey Bootsma

Zooplankton represent a critical component of aquatic food webs in that they transfer energy from primary producers to higher trophic positions. However, their small size makes the application of traditional trophic ecology techniques difficult. Fortunately, novel techniques have been developed that can be used to elucidate feeding information between zooplankton species. I used the analysis of stable carbon and nitrogen isotope ratios to estimate the trophic structure of Lake Michigan's zooplankton community. The major zooplankton species, three size classes of seston, and seston from specific water column depths were collected in 2011 and 2012 for stable isotope analysis. Trophic position was estimated for the major zooplankton species by relating their mean nitrogen stable isotope signatures to the assumed primary consumer *Daphnia mendotae*. Initial results suggested that the large calanoid copepod *Limnocalanus macrurus* occupies a trophic position of a tertiary consumer, nearly a full trophic position above all other copepods species.

In 2013, subsequent sampling and analyses were done to determine if the high nitrogen stable isotope signature for *L. macrurus* was the result of trophic enrichment or some

other mechanism. Zooplankton and seston samples were collected in 2013 from winter through summer. A seasonal trend in the stable nitrogen isotope ratios was observed in seston, with high values during the winter and low values during the summer. A dynamic model was developed and used to estimate the effects of this seasonal trend on the nitrogen stable isotope signature of *L. macrurus* and two other copepod species. The model results suggest that *L. macrurus* as well as two other copepods are reacting quickly to changes in the stable isotope signature of seston. Therefore, some other mechanism is likely causing the $\delta^{15}\text{N}$ signature of *L. macrurus*.

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Chapter 1: Introduction and Background

1.1 Food Webs and Stable Isotope Analysis

One of the central themes of ecology is the understanding of the flow of energy within natural systems. This information is not only important from a scientific perspective but also helps to better manage natural resources. The feeding interactions between all organisms within a system are described using the concept of a food web. Early depictions of food webs are recorded as early as 1923 when Summerhayes and Elton published a conceptual depiction of how nitrogen is transferred between organisms on Bear Island, Norway (Morin 1999). However, in 1942 Raymond Lindeman largely popularized the food web concept. In his milestone paper “The Trophic-Dynamic Aspect of Ecology” Lindeman described feeding interaction in terms of energy transfer. Specifically, he postulated that energy transfers between trophic positions are relatively inefficient with the majority of energy lost between trophic transfers (Lindeman 1942). This places constraints on the number of trophic transfers that can occur within a system limiting the maximum trophic position of the food web. This ecological efficiency hypothesis is central to food web theory and has largely paved the way for the work of future ecologists.

Conventionally, ecologists attempt to reconstruct food webs by combining information on interactions between organisms and nutrients to build conceptual or mathematical models (Morin 1999). Because mapping interactions between all organisms is difficult if not impossible, ecologists tend to group taxonomically or behaviorally similar organisms into feeding clades called trophic positions. In the simplest situation, models can use a linear food chain by placing primary consumers at the base followed by herbivores and carnivores. Alternatively, much more complex models can exist that may further divide trophic positions into subgroups, include feeding strategies like omnivory, or take into account life history and temporal and spatial variability of feeding behaviors. The complexity of the model will depend on the questions addressed and components of the system that need to be included. Traditionally, ecologists have used observational techniques to

determine feeding interactions between organisms within the environment. Some of these techniques include gut content analysis, the study of morphological characteristics, food preference studies, or direct observations (Warren 1985; Wong & Chow-Fraser 1985; Vanderploeg, Liebig & Omair 1993; Martin & Cash-Clark 1995). However, these techniques have been criticized as being inhibited by the researcher's inability to fully understand the true complexity of the system (Paine 1988; Polis 1991). Because of this, food web theory has been criticized for being an oversimplification of a complicated system (Paine 1988; Polis 1991).

Fortunately, in the last few decades chemical techniques have been developed that have the potential to allow researchers to gain a fuller understanding of both food web structure and function. These techniques are more objective and are able to better identify spatial and temporal trends that traditional methods could not. One such technique that is increasingly being applied to help determine trophic relationships between organisms is the analysis of the carbon and nitrogen stable isotope.

Stable isotope analysis is done by examining ratios of heavy to light stable isotopes within a sample. To help describe these isotopic ratios, scientists have developed standard notations. The notation most often used to describe the amount of heavy to light isotopes in a sample is the δ (delta) notation. The δ notation is the ratio of light to heavy isotopes of a sample measured relative to a standard. The equation used to calculate the δ value of a sample is found below:

$$\delta^{15}N \text{ or } \delta^{13}C = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] * 1000 \quad (\text{Equation 1})$$

where R is the $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ of the sample or standard. Units used for the δ notation are “per mil” (‰). Standards will have a δ value equal to 0‰ (Fry 2006).

Stable isotope analysis relies on the fact that in kinetic chemical reactions, the lighter stable isotope is favored over the heavy stable isotope. This results in a higher concentration of the lighter stable isotope in the product. The separation of the heavy and light stable isotope in a process is commonly termed fractionation (Fry 2006). Ecologists exploit this phenomenon to explain processes by making general assumptions about these fractionations within organisms or within the environment (Peterson & Fry 1987). One of the more common uses for stable isotope analysis is to determine feeding relationships between organisms. This method relies on the relatively consistent fractionation of both the nitrogen and carbon stable isotopes during trophic transfers (Peterson & Fry 1987). Based on the results of empirical observations, it is generally assumed that the nitrogen stable isotope is fractionated 3.4 ‰ at each trophic transfer (Minagawa & Wada 1984; Vander Zanden & Rasmussen 2001; Post 2002), although it can range from -0.7 to 9.2‰ (Vander Zanden & Rasmussen 2001). As a result, an organism's $\delta^{15}\text{N}$ signature is usually an indication of its trophic position, i.e. how many trophic transfers occur between it and the base of the food web. Conversely, the carbon stable isotope is generally fractionated much less during trophic transfers. In this case, the consumer's stable isotope signature will become heavier or more “enriched” compared to the food source by approximately 1 ‰ (Peterson and Fry 1987). Since the carbon stable isotope ratio is more conserved during trophic transfers it is generally used to determine the carbon source at the base of the web. Therefore, by simultaneously analyzing the carbon and nitrogen isotope, ecologists can gain information on both the food source and trophic position of an organism.

Stable isotope analysis has several benefits over traditional techniques. First, unlike other techniques that only provide a snap shot of what an organism is eating, the stable isotope signature of an organism's tissue provides a time integrated value of a consumer's diet (Fry &

Arnold 1982; Hesslein, Hallard & Ramlal 1993; MacAvoy, Macko & Garman 2001; MacAvoy, Macko & Arneson 2005). Secondly, stable isotope analysis can reveal the relative importance of food sources like detritus or phytoplankton that are difficult to identify visually (Mulholland *et al.* 2000). Finally, this technique can be applied to small organisms for which gut contents may be difficult to identify (Ngochera & Bootsma 2010).

1.2 The Role of Zooplankton in Lake Food Webs

Zooplankton are organisms that live in the pelagic water column and are incapable of swimming against the current. These animals can range in both size and taxonomy. The species diversity of zooplankton in freshwater systems is much smaller than that in marine systems and is generally made up of four major groups, including protists, rotifers, cladocerans, and copepods (Wetzel 2001). These organisms are small, ranging in size from a few microns to a few millimeters in length, with protists and rotifers generally being much smaller than cladocerans and copepods.

Despite their small size, zooplankton play a major role in aquatic food webs. By feeding on microscopic organisms like phytoplankton and protozoa, crustaceous zooplankton occupy a critical position in the food web, linking the lower food web to higher trophic position organisms such as fish (Wetzel 2001). Additionally, through grazing zooplankton can effectively shape the composition and control the biomass of the lower trophic positions (Carpenter, Kitchell & Hodgson 1985). Finally, zooplankton are important nutrient recyclers. Zooplankton excrete both phosphorus and nitrogen, therefore returning nutrients to lower trophic positions (Korstad 1983; Gulati, Perez Martinez & Siewertsen 1995). Thus, understanding trophic links between zooplankton species is an important pursuit in aquatic ecology.

However, the small size of zooplankton species makes traditional techniques used to determine trophic interaction difficult to apply. Because of this, plankton ecologists have historically relied on food preference studies and swimming behavior of zooplankton to infer

feeding relationships between animals in the environment (Wong & Sprules 1984; Warren 1985; Vanderploeg *et al.* 1993). Yet, these methods rely on the assumption that organisms behave the same way in the environment as they do in the laboratory. To help validate this assumption, contemporary techniques like stable isotope analysis can be applied to help build confidence in our understanding of the trophic relationships of zooplankton in aquatic systems.

1.3 The Changing Lake Michigan Food Web

The Ponto-Caspian zebra mussel (*Dreissenid polymorpha*) was first discovered in Lake Michigan in 1989 (Nalepa *et al.* 2006). Between 1992 and 2002, *D. polymorpha* rapidly colonized nearshore regions in Lake Michigan (Nalepa *et al.* 2006). In 1997, the closely related *Dreissena rostriformis bugensis* (quagga mussel) was first identified in Lake Michigan (Nalepa *et al.* 2001). These mussels quickly began replacing *D. polymorpha* at sites already colonized. In 2002, *D. bugensis* began to expand to depths between 50-90m, and by 2005, had reached estimated average lake wide densities of 8816 individuals/m² (Nalepa & Fanslow 2010).

These mussels have had unprecedented effects on the Lake Michigan ecosystem. Their offshore spread has been linked to a population decline of the benthic invertebrate *Diporeia* (Nalepa, Fanslow & Lang 2009). *Diporeia* is a deep water amphipod that historically played a keystone role in Lake Michigan's food web by transferring primary production to higher trophic levels (Gardner *et al.* 1990). Between 1994 and 2005 the populations size of *Diporeia* decreased by 96% in regions with a depth between 30-90m (Nalepa *et al.* 2009). Planktivorous fish biomass also decreased during this time (Bunnell *et al.* 2013). This low prey fish biomass has persisted over the last five years, with the lowest biomass recorded for Lake Michigan by the United States Geological Survey (USGS) in 2012 (Bunnell *et al.* 2013).

Dreissenid mussels are also thought to have a major impact on the nutrient cycling in the lake. In 2004, Hecky *et al.* (2001) presented their now well cited hypothesis "The

Nearshore Energy Shunt”. This conceptual model hypothesized that nearshore dreissenid mussels are concentrating phosphorus inputs at the lake bottom in the nearshore zone, thus reducing the amount of nutrients available to move. A similar hypothesis was later presented suggesting that this same phenomenon was occurring in areas with a depth of 30-50m following the offshore spread of *D. bugensis* (Vanderploeg *et al.* 2010).

Additionally, oligotrophication of offshore waters has recently been reported. Offshore chlorophyll concentrations and primary production were significantly lower during the 2007 and 2008 spring isothermal period than in pre-dreissenid years. During lake stratification, however, chlorophyll concentrations and primary production were not significantly different from pre-dreissenid years. The authors of this study suggested that because the water column is well mixed during the spring isothermal period, offshore mussels have access to the full water column. This allows dreissenid mussels to remove large amounts of phytoplankton from the water column (Fahnenstiel *et al.* 2010).

This loss of a major energy source could be contributing to some of the changes recently observed in Lake Michigan’s zooplankton community. Soon after the offshore spread of *D. bugensis*, the total zooplankton biomass in Lake Michigan began to decline. The major daphnid species, *Daphnia mendotae*, represented a significant portion of this loss of biomass (Barbiero *et al.* 2009). Cyclopoid copepod populations are believed to have crashed (Vanderploeg *et al.* 2012). *Leptodiaptomus minutus*, *Leptodiaptomus ashlandi*, and *Mysis relicta* have also experienced a loss of biomass (Pothoven, Fahnenstiel & Vanderploeg 2010; Vanderploeg *et al.* 2012). Similar trends were observed in Lake Huron’s zooplankton community following the spread of dreissenid mussels (Barbiero & Tuchman 2004b). Conversely, many large species of calanoid copepods have increased in biomass. *Limnocalanus macrurus* is estimated to contribute 60% to the total summer zooplankton biomass in Lake Michigan (Barbiero *et al.* 2009). Other large calanoids including *Leptodiaptomus sicilis*, and *Epischura lacustris*, and the predatory

cladoceran *Bythotrephes longimanus* (hereafter *Bythotrephes*) have also recently exhibited more modest population increases (Barbiero *et al.* 2009; Vanderploeg *et al.* 2012).

The mechanisms and implications for these biomass and species composition shifts in the zooplankton community are poorly understood. A clearer understanding of the trophic dynamics of zooplankton communities in general is required to help better answer these questions. Information on trophic interactions between zooplankton has historically been based on feeding relationships observed in food preference studies (Warren 1985; Burns & Gilbert 1993; Liebig & Vanderploeg 1995). Assumed trophic interactions have been used to generate simple to complex food webs (Sprules & Bowerman 1988). Although helpful, these traditional food webs have been criticized as being subjective illustrations based on the inability of researchers to fully document the complex temporal, spatial, and taxonomic variations in actual ecosystems (Paine 1988; Polis 1991). This is especially true for small, difficult to study organisms like zooplankton, where trophic lumping of taxonomic groups is common (Polis 1991). Fortunately, techniques are now available that can help to integrate temporal, spatial, and taxonomic variations in ecosystems into food web studies. The application and advancement of these techniques are critical in answering complex ecological questions and helping better manage aquatic systems.

Chapter 2: Zooplankton Trophic Dynamics within Lake Michigan Revealed Using the Analysis of the Carbon and Nitrogen Stable Isotopes and Biomass Measurements

2.1 Chapter Objectives

To better understand zooplankton trophic dynamics in Lake Michigan, stable isotope ratios of carbon and nitrogen were analyzed for the major zooplankton species. Specific aims of this were to: (i) estimate trophic positions of the dominate zooplankton species, (ii) compare stable isotope results to published feeding behaviors, (iii) determine relative importance of species through biomass estimates, (iv) determine spatial and size related trends in the carbon and nitrogen isotopes of seston.

2.2 Methods

2.2.1 Sample Collection and Processing

On seven dates between June and October 2011, zooplankton samples were collected from an offshore site ($z=50-70\text{m}$) in southwest Lake Michigan. Samples were collected using nets with a mesh size of $210\text{ }\mu\text{m}$ and 1mm by lowering the nets to approximately one meter above the lake bottom. Water samples were collected from a depth of 15m and 35m , pooled, and assumed to represent the upper water column. All water samples were collected using a 4-L Niskin Bottle.

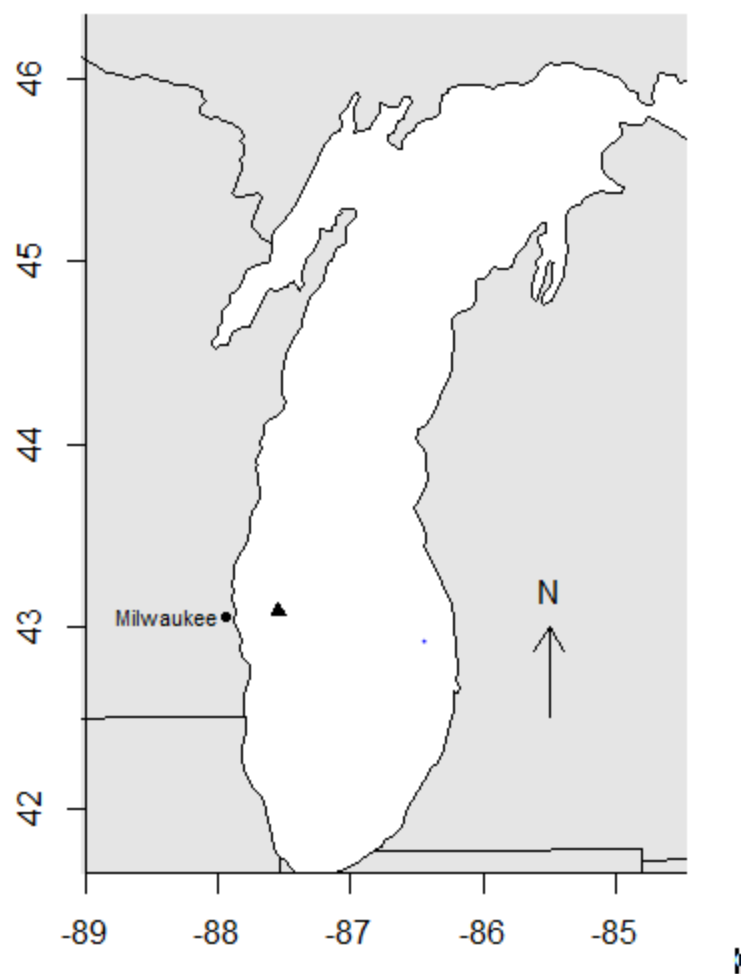


Figure 1: Location of zooplankton and seston sample collection in 2011, 2012, and 2013. In 2011, zooplankton and seston were collected at a depth between 50-70m while in 2012 they were collected exclusively from the 70m site (black triangle). The coordinates of this sampling site were 43° 4' 22.08" N" and 87° 47' 24.

Water samples were brought back to the lab and processed immediately. Water was passed through a 210 μ m filter to remove most large zooplankton. Each water sample then went through a series of filtrations to create three different particle size classes. Because non-living particles may have made a contribution, these samples were defined as seston. However, it is assumed that the majority of particles within the samples were made up of living material. The three seston size classes within this study were microplankton (64-210 μ m), nanoplankton (3-64 μ m), and picoplankton (<3 μ m). The three seston size classes were individually filtered onto

ashed GF/F filters. The GF/F filters were rinsed with 5M HCl in order to remove any inorganic carbon and then rinsed with distilled water (DeNiro & Epstein 1978). The samples were placed in a desiccator and allowed to dry completely before being packed in aluminum foil disks for stable isotope analysis.

Following collection, zooplankton were allowed to sit for several hours to evacuate the content of their guts before being narcotized with carbonated water. Using a dissecting scope and a Bogorov Counting Chamber, major zooplankton species in each sample were identified to the lowest taxonomic level possible, enumerated, and handpicked onto GF/F filters. Copepodites and adults were not separated for analysis. Depending on the size of the plankter, approximately 50-200 individuals were placed onto each filter. Samples were acidified using 5% HCl to remove inorganic carbon that may have been left behind from the carbonated water (DeNiro & Epstein 1978). Filters were placed into a desiccator to dry completely and then were packed in aluminum foil disks for stable isotope analysis.

Seston and zooplankton samples were collected again between 5/22/2012 and 11/12/2012. Samples were collected exclusively at the 70m site. To determine daytime depth preference of major zooplankton species, depth specific sampling was conducted. A closing zooplankton net was constructed using a release mechanism removed from an old Niskin bottle. A detailed description of how the net was constructed can be found in Appendix 1. Before collecting any samples, a Seabird Conductivity, Temperature, and Depth (CTD) sensor was sent to the lake bottom. Aboard the ship, the data was downloaded and the depth of the thermocline was determined. Based on this, the depths of epilimnion, metalimnion, and hypolimnion were estimated. The closing zooplankton net was used to sample these three water column layers. Water samples were also collected from the three water column depths using a 4-L Niskin bottle. The Niskin bottle was used to sample water from the middle depth of each water column layer.

Zooplankton samples were processed using a similar procedure as was done in 2011.

Unlike 2011, seston samples were not separated by size class. Water samples collected from the three water column depths were filtered onto individual GF/F filters, acid washed, and packed for stable isotope analysis.

2.2.2 Stable Isotope Analysis

Stable isotope measurements were made on an isotope ratio mass spectrometer (Finnigan MAT delta S SIR-MS, with elemental analyzer front end and ConFlo II interface). Carbon calibration was done with NIST standard RM 8542 (sucrose, $\delta^{13}\text{C}=-10.47$) and a NIST-traceable standard (glycine, $\delta^{13}\text{C}=-33.63$). Nitrogen calibration was done with NIST standard RM 8547 (IAEA-N1 ammonium sulfate, $\delta^{15}\text{N}=0.4$), NIST standard RM 8548 (IAEA-N2 ammonium sulfate, $\delta^{15}\text{N}=20.3$), and a NIST-traceable ammonium chloride standard ($\delta^{15}\text{N}=-8.9$). During sample runs, an acetanilide control sample was run every twelfth sample and analyzed for $^{13}\text{C}:^{12}\text{C}$ and $^{15}\text{N}:^{14}\text{N}$ ratios. $^{13}\text{C}:^{12}\text{C}$ ratios ($\delta^{13}\text{C}$ values) were determined relative to the PDB carbonate standard, and $\delta^{15}\text{N}$ values were determined relative to the $^{15}\text{N}:^{14}\text{N}$ ratio of air. Isotope ratios are expressed as per thousand (‰) differences between the isotope ratio of the sample and that of the standard, using equation 1.

2.2.3 Lipid extraction and Correction

Bulk zooplankton samples were collected to determine the degree to which carbon isotope ratios were affected by lipid content, as lipids tend to be less ^{13}C -enriched than other tissues (Peterson & Fry 1987). Lipids were extracted using 2:1 chloroform methanol solution. Solutions were centrifuged and decanted a total of three times. Lipid content was calculated gravimetrically and the C and N content of lipid extracted tissue was measured using gas chromatography preceded by sample combustion. This allowed determination of the relationship between zooplankton lipid content and C:N ratio. $\delta^{13}\text{C}$ signatures of zooplankton samples were then plotted against their C:N. The slope of the regression fitted through the plot (slope=1.418, $R^2=0.87$) and the C:N ratio

(3.8) of the lipid extracted bulk zooplankton sample were then used to develop a lipid correction equation for $\delta^{13}\text{C}$ of zooplankton samples (Smyntek *et al.* 2007):

$$\delta^{13}\text{C}_{corrected} = \delta^{13}\text{C}_{corrected} + 1.417 * (C:N_{sample} - 3.8) \quad (\text{Equation 2})$$

2.2.4 Biomass Estimates

Individual zooplankton species were sorted onto GF/F filters, dried in a desiccator, and packed into aluminum foil discs. The C and N content of each sample were then determined using the gas chromatograph on the front end of the SIR-MS. Next, the average mass of an individual zooplankton for each sample was calculated by dividing the mass of the entire sample by the number of individual zooplankton that were sorted onto the GF/F filter. Individual zooplankton species in a sample were enumerated using a Bogorov Counting Chamber. The volume of the water column filtered was determined by multiplying the area of the neck of the net by the depth of the water column. This information was used to calculate zooplankton species concentration as individuals L^{-1} . Finally, the average mass of an individual was multiplied by the zooplankton taxon concentration in the water column to determine biomass as $\mu\text{g C L}^{-1}$ for each taxon.

Seston biomass was also expressed in units of $\mu\text{gC/L}$. As for the zooplankton samples, the mass of each filter in units $\mu\text{g C}$ was determined using a gas chromatograph. The mass of the sample was then divided by the volume of water filtered in order to get the biomass of the sample.

2.2.5 Trophic Position Estimation

Due to fast population turnover rates of phytoplankton (which are assumed to make up the majority of seston in pelagic samples), their $\delta^{15}\text{N}$ signature is assumed to respond rapidly to isotopic changes in nutrient sources (Cabana & Rasmussen 1996; Woodland *et al.* 2012).

Therefore, using a relatively short time series of seston $\delta^{15}\text{N}$ signatures as indicators of the base of the food web can be misleading because any change in $\delta^{15}\text{N}$ in the nitrogen pool will rapidly be reflected in their tissue (Cabana & Rasmussen 1996; Post 2002). The standard method for trophic position estimation of a consumer is to express its $\delta^{15}\text{N}$ signature relative to that of a primary consumer that reacts at a similar rate to changes in the $\delta^{15}\text{N}$ of primary producers (Cabana & Rasmussen 1996; Vander Zanden & Rasmussen 2001; Post 2002). By assuming a nitrogen fractionation factor per trophic transfer ($\Delta^{15}\text{N}$), and that the primary consumer occupies a trophic position of 2, the trophic position of a consumer can be estimated using the equation:

$$\text{Trophic Position} = \frac{(\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{Primary Consumer}})}{\Delta^{15}\text{N}} + 2 \quad (\text{Equation 3})$$

The fractionation factor, $\delta^{15}\text{N}$, is generally assumed to be equal to 3.4 ‰ (Minagawa & Wada 1984; Vander Zanden & Rasmussen 2001; Post 2002). For larger organisms in aquatic systems, mussels are commonly used as an assumed primary consumer. However, because zooplankton react more quickly to changes in the $\delta^{15}\text{N}$ of seston, *Daphnia* has been suggested to be used as a primary consumer for zooplankton trophic estimation using stable isotopes (Matthews & Mazumder 2003). Therefore, *D. mendotae* was used as the assumed primary consumer in this study. Mean trophic positions of each organism were then calculated using *D. mendotae* as a reference.

2.2.6 Biomass Pyramids

The distribution of biomass within a food web can exist in several forms. Typically, the biomass of a food web is distributed like a pyramid; biomass decreases with increasing trophic position. Although, biomass can be distributed in other forms including spindle shaped and inverted pyramids (Morin 1999). To determine the distribution of biomass in the food web each zooplankton species was grouped as a primary, secondary, or tertiary consumer by rounding their trophic position to the nearest whole number. For example, a zooplankton species with a mean trophic position of 3.3 would be considered a secondary consumer. The average 2011 biomass for each group was summed and plotted. The average 2011 biomass of each of the three seston size classes were plotted below secondary consumer biomass in ascending order of their mean $\delta^{15}\text{N}$ signatures.

2.3 Results

2.3.1 Biomass and Abundance

Picoplankton, nanoplankton, and microplankton contributed to the mean seston biomass in descending order. All three size classes were found to have relatively high biomass on 6/30/2011. However, during July, microplankton biomass dropped considerably, while the other two size classes remained stable. Like zooplankton, total seston followed a seasonal trend, reaching a peak biomass on 8/30/2011. Microplankton contributed the most to this peak, though picoplankton also had its maximum biomass at this time (Fig. 1). Both epilimnetic seston and metalimnetic seston had similar biomass in 2012. Hypolimnetic seston biomass was slightly under half the amount of the other two water column depths. All three depths had the highest biomass in late June (Fig. 2).

Copepods made up the bulk of the total zooplankton biomass, consistently contributing substantially more than cladoceran species (Fig 3). The three copepods that contributed the most to the total biomass were *Leptodiaptomus spp.*, *Limnocalanus macrurus*, and *Diacyclops thomasi*.

The biomass of *D. thomasi* was higher in early summer, though it decreased as the season moved on. *Bythotrephes* had the highest mean biomass of all cladocerans sampled, though still much lower than copepod biomass. Total zooplankton biomass followed a seasonal trend, with values increasing in July, reaching a maximum in late August, and decreasing again in fall (Fig. 3). Likewise, zooplankton abundance followed similar patterns with *Leptodiatomus* having the highest concentration. *L. macrurus* was not as abundant as the other copepods, but it made up a large proportion of the biomass, due to its large size.

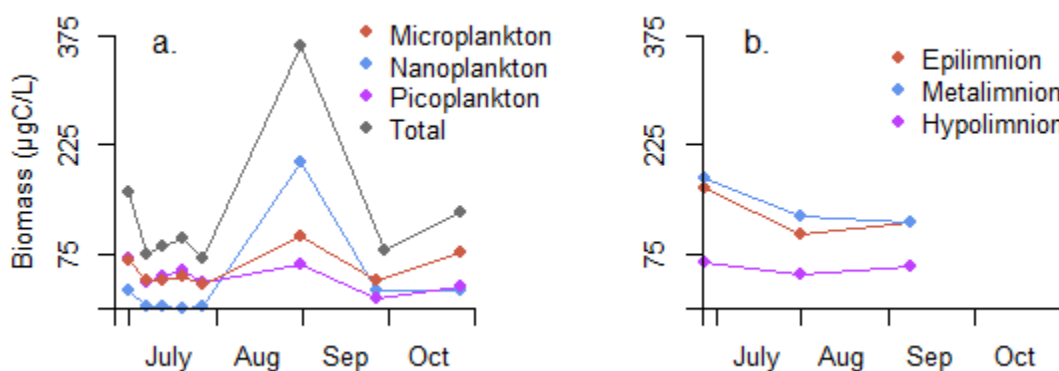


Figure 2: Biomass estimates for three seston size classes collected in 2011 (a.) and bulk seston biomass estimates at three different water column depths collected in 2012 (b.).

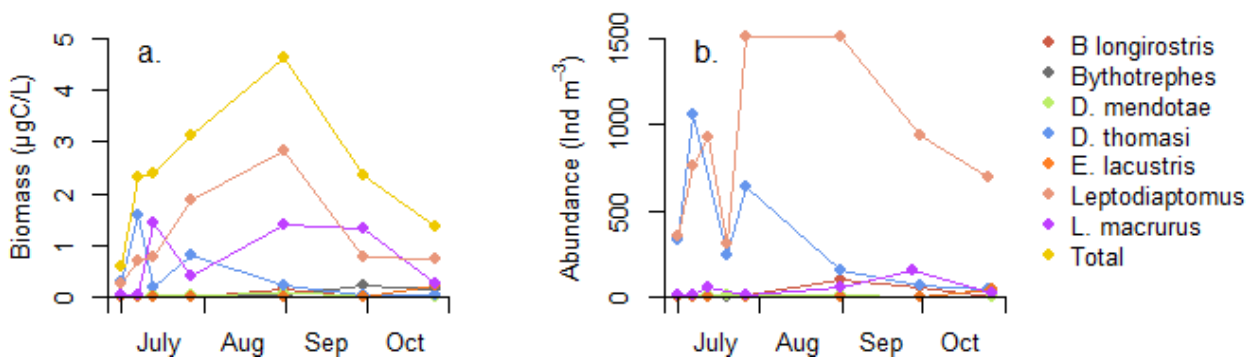


Figure 3: Biomass (a) and abundance (b) of the major species of zooplankton in Lake Michigan collected in 2011.

2.3.2 Depth Specific Zooplankton Sampling

The three *Leptodiaptomus* species had varying depth preferences, with *L. sicilis* more evenly distributed throughout the water column than the other two species. The predatory cladocerans *Bythotrephes longimanus* and *Leptodora kindti* were found exclusively in the epilimnion. *D. mendotae* occupied both the epilimnion and hypolimnion in almost even abundances. The large calanoid *L. macrurus* was found showed a greater preference for the hypolimnion than any other species sampled.

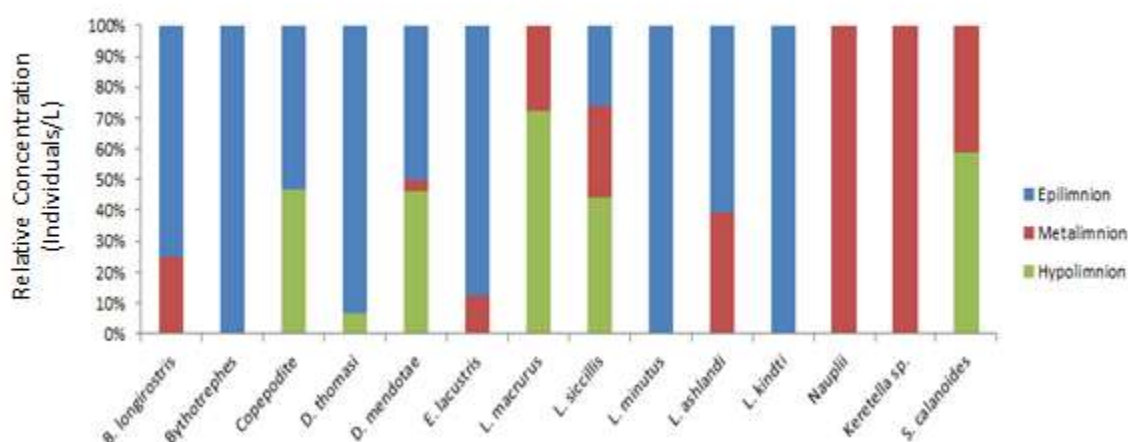


Figure 4: Percent concentration (individuals L⁻¹) of major zooplankton species within specific water column depths relative to total abundance for that species. Samples were collected in early June, late July, and early September of 2012. The bars represent an average percent abundance for all three time periods.

2.3.3 Stable Isotope Signature and Trophic Positions

Nitrogen stable isotope signatures revealed several trophic levels among the zooplankton species (Fig. 5). The cladocerans *D. mendotae* and *Bosmina longirostris* had low $\delta^{15}\text{N}$ signatures near 3‰. Most copepods, along with *Bythotrephes*, on average were enriched between 2.88 ‰ and 3.91‰ compared to *D. mendotae*. *L. macrurus* had the highest mean $\delta^{15}\text{N}$ signature—at least 3.5‰ greater than all other copepod species. Trophic estimations followed the same pattern as

$\delta^{15}\text{N}$ signatures with *L. macrurus* occupying the maximum trophic position of 4.24 and *D. mendotae* occupying the lowest trophic position (Appendix 2).

Like the nitrogen stable isotope, the mean carbon stable isotope showed a similar yet less dramatic stepwise enrichment with trophic position. A simple linear regression between the mean carbon and nitrogen stable isotope signatures of all seston and zooplankton shows a statistically significant regression line with a slope of 0.3584 ($R^2 = 0.5879$, $p\text{-value} = 0.00223$). Assuming a 3.4‰ trophic fractionation factor for the nitrogen stable isotope, a trophic transfer results in a ^{13}C enrichment of 1.19‰. *L. macrurus* had the highest mean $\delta^{13}\text{C}$ of all samples. The mean carbon stable isotope signatures for *B. longimanus* and *D. mendotae* were similar to each other and enriched compared to all seston samples. No significant differences were found between any of the seston size classes' stable nitrogen isotope signatures (Appendix 3). Alternatively, nanoplankton's carbon stable isotope signature was heavier and significantly different from both microplankton and picoplankton (Appendix 3).

In 2011, relatively high nitrogen stable isotope values were observed in June and July for both zooplankton and seston samples (Fig. 6). Many of these signatures dropped rapidly into July while others remained elevated. Specifically, *L. macrurus* retained a high $\delta^{15}\text{N}$ signature during the month of July before dropping by about 6‰ between July and late September. A similar seasonal trend was observed in 2012 for *D. mendotae* and *Leptodiaptomus*. High $\delta^{15}\text{N}$ values were observed in early June followed by a summer low. Following the summer minimum, the $\delta^{15}\text{N}$ signature of these species, along with *Bythotrephes* and *E. lacustris*, increased into the fall. Unlike in 2011, the signature for *L. macrurus* did not have an early summer maximum in 2012, though it was higher than all other species sampled during July, August, and September.

Microplankton $\delta^{13}\text{C}$ signature drops sharply in July of 2011 and remains less enriched than all zooplankton species until late October (Fig. 7). Likewise, picoplankton's $\delta^{13}\text{C}$ is below that of all zooplankton species until October where it becomes more enriched than

Leptodiaptomus. In 2012, seston again had generally less enriched $\delta^{13}\text{C}$ stable isotope signatures than zooplankton species. With the exception of one point in late July, *L. macrurus* had a more enriched $\delta^{13}\text{C}$ stable isotope signature than all zooplankton sampled in 2012. In 2012, hypolimnetic seston had the highest mean $\delta^{15}\text{N}$ signatures, while epilimnetic seston had the most enriched mean $\delta^{13}\text{C}$ signatures

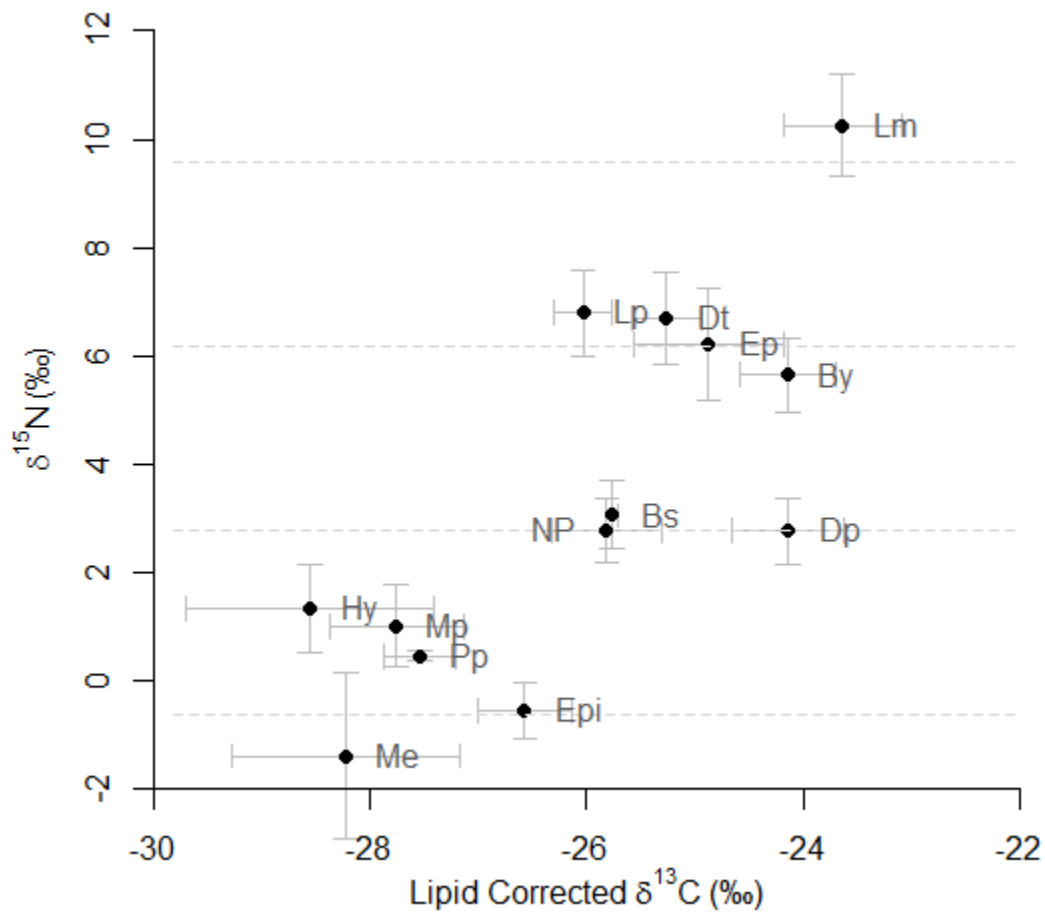


Figure 5: Average $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures of zooplankton and seston samples collected in 2011 and 2012. Confidence intervals represent standard errors. Dashed lines represent trophic positions based on an assumed 3.4‰ fractionation factor and that *D. mendotae* is a primary consumer. Actual values can be found in Appendix 2. Labels are as follows: **Me**: metalimnetic seston, **Epi**: epilimnetic seston, **Hy**: Hypolimnetic seston, **Pp**: Picoplankton, **Mp**: Microplankton, **NP**: Nanoplankton, **Bs**: *B. longirostris*, **Dp**: *D. mendotae*, **By**: *Bythotrephes*, **Ep**: *E. lacustris*, **Dt**: *D. thomasi*, **Lp**: *Leptodiaptomus*, **Lm**: *L. macrurus*

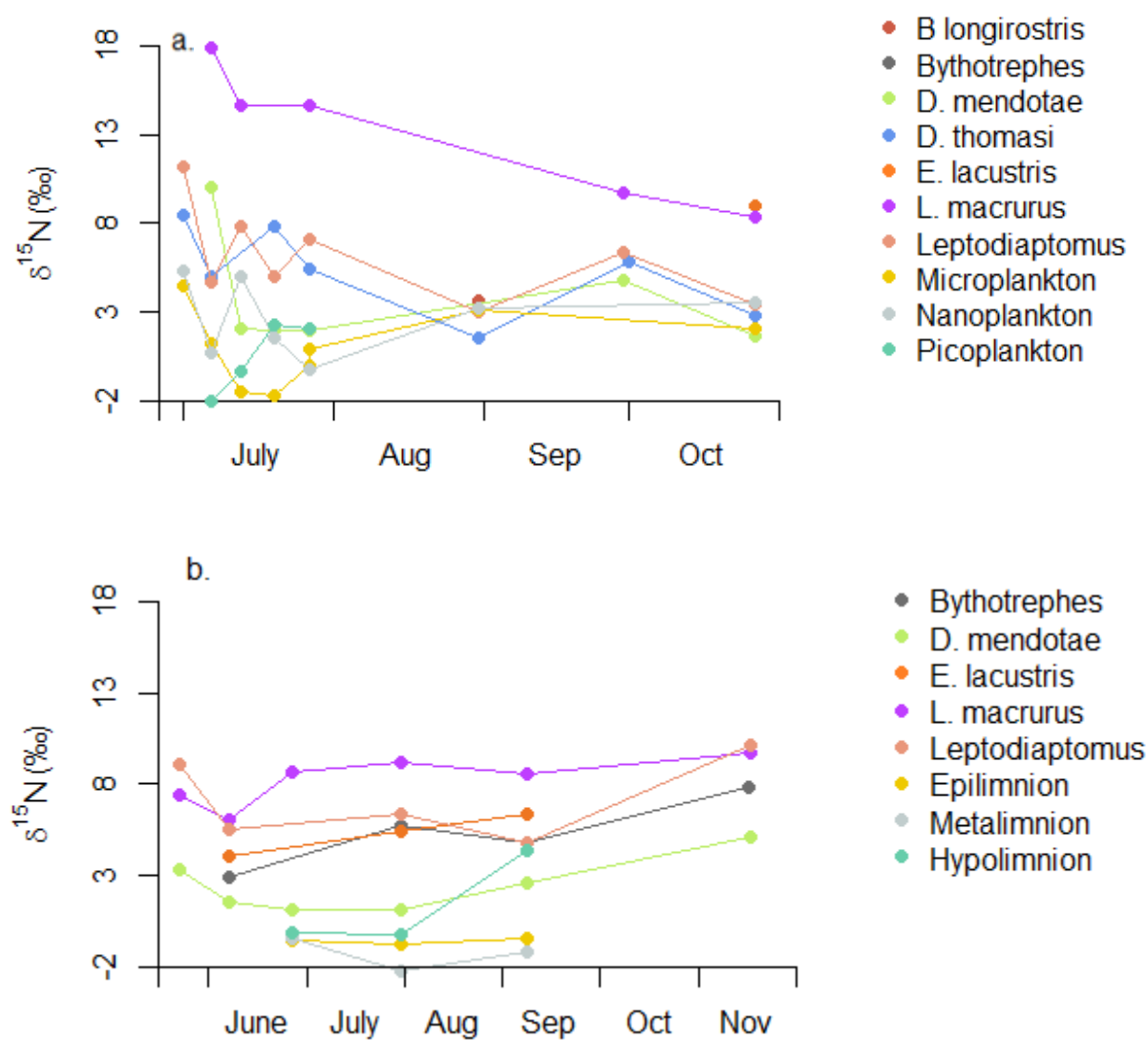


Figure 6: Stable nitrogen isotope signatures of major Lake Michigan zooplankton and three seston size classes collected in 2011 (a) and zooplankton and depth-specific seston samples collected in 2012 (b).

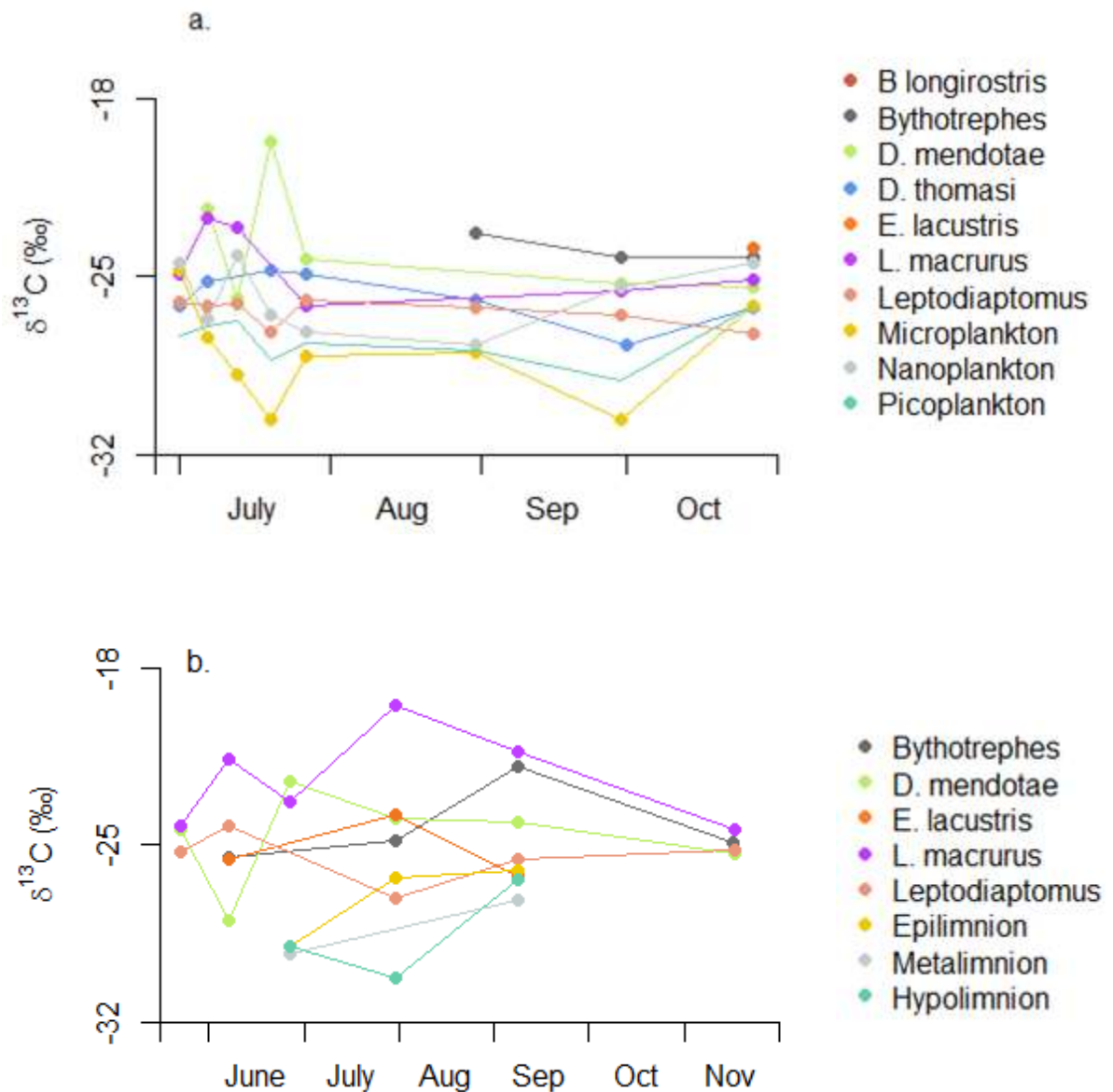


Figure 7: Stable carbon isotope signatures of major Lake Michigan zooplankton and three seston size classes collected in 2011 (a) and zooplankton and depth specific seston samples collected in 2012 (b).

2.3.4 Biomass Pyramid

Zooplankton Species were organized into trophic groups using their mean trophic positions and plotted against the sum of each group's biomass. *D. mendotae* were the sole primary consumers, while *Leptodiaptomus*, *D. thomasi*, *E. lacustris*, *Bythotrephes*, and *B. longirostris* were

considered secondary consumers. *L. macrurus* alone was estimated to be a tertiary consumer.

Primary consumers' biomass was two to three orders of magnitude smaller than tertiary consumers' and secondary consumers' respectively (Fig. 9). Because seston biomass was much higher than the total zooplankton biomass, they were removed from the first biomass pyramid to better show trends in the zooplankton community. The biomass was considerably higher for all three size classes of seston than for zooplankton (Fig. 10).

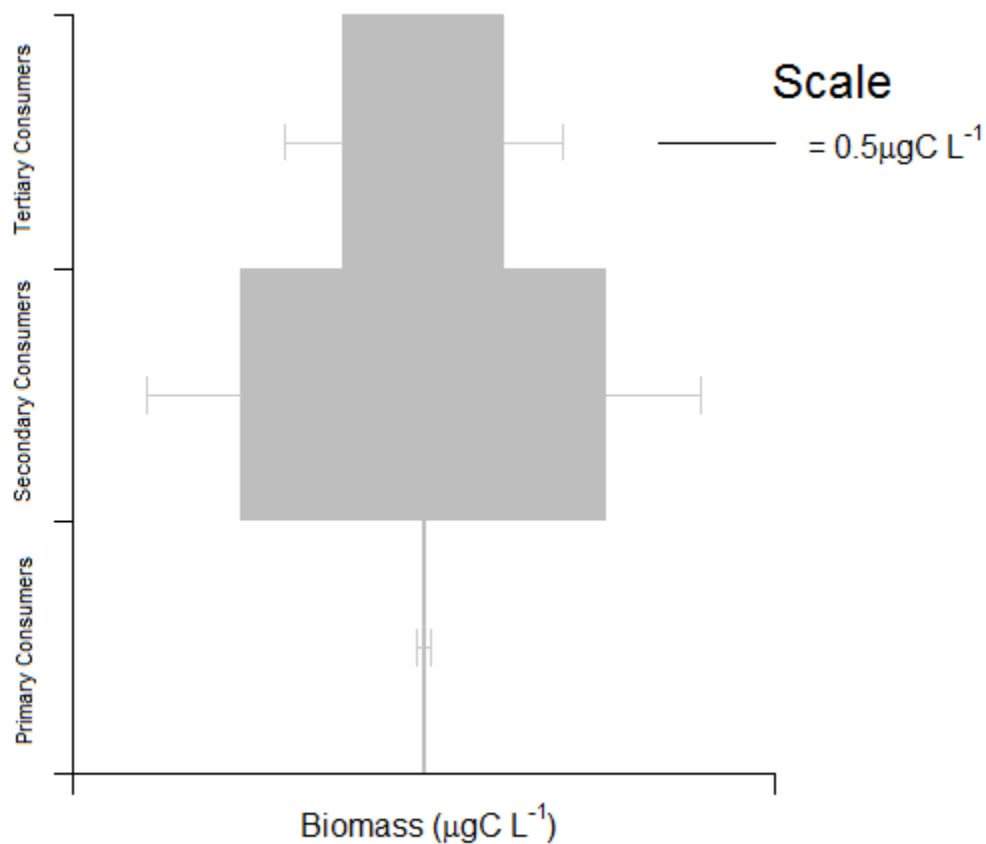


Figure 8: Biomass pyramid of major zooplankton species in Lake Michigan. Consumers were separated into groups by rounding their mean trophic position to the nearest whole number. Biomass for each species is the sum of each group's mean 2011 biomass. Error bars represent the standard deviation of each group.

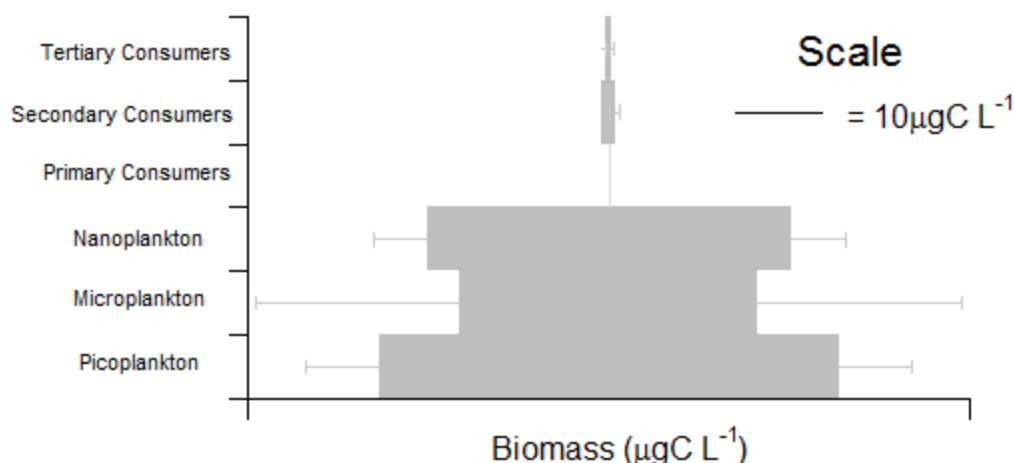


Figure 9: Biomass pyramid of major zooplankton species and three size classes of seston in Lake Michigan. Consumers were separated into groups by rounding their mean trophic position to the nearest whole number. Biomass for each species is the sum of each group's mean 2011 biomass. Seston size classes were organized in ascending order of $\delta^{15}\text{N}$ signature. Primary consumer biomass was too small to be represented on the plot so a small line was plotted between secondary consumers and nanoplankton to represent connectivity between trophic groups.

2.4 Discussion

2.4.1 Zooplankton Abundance and Biomass

Abundance estimates made in 2011 were much lower than historic estimates made in Lake Michigan. . Lake-wide abundance for both the spring mixing and summer stratified periods between 1983-1992 for *Leptodiatomus* adults and copepodites were on average 6346 individuals m^{-3} (Makarewicz *et al.* 1995). Estimates of total *Leptodiatomus* abundance in 2011 was only about 13.9% of this total. Likewise, *D. thomasi* estimates in 2011 were approximately 16.5% of historical values, while *D. mendotae* abundance was less than one percent of their historical lake-wide abundance (Makarewicz *et al.* 1995). The low zooplankton abundance in 2011 may represent a continuation of a trend of decreasing zooplankton in Lake Michigan. In 2002, *D. bugensis* began to expand to depths between 50-90m, and by 2005, had reached estimated average lake-wide densities of 8816 individuals/ m^2 (Nalepa *et al.* 2009). Following this expansion, total

zooplankton biomass declined sharply, with herbivorous cladocerans making up much of this decline (Barbiero *et al.* 2009; Barbiero, Lesht & Warren 2012; Vanderploeg *et al.* 2012).

Interestingly, an increase in the abundance of *L. macrurus* was observed at this time (Barbiero *et al.* 2009; Doubek & Lehman 2011). Though the 2011 abundance estimates for *L. macrurus* were still less than historic estimates, they represent only a 12.7% decrease (Makarewicz *et al.* 1995).

Because most published zooplankton biomass estimates are expressed in units of μg dry weight (DW), it is difficult to compare the 2011 biomass estimates to other published values without knowing the contribution of carbon to the total mass of each zooplankton sample. This becomes increasingly difficult because no information was taken on life stage, and the carbon content of zooplankton can change as individuals increase in size (Ch  telat, Amyot & Cloutier 2012). However, zooplankton carbon content typically ranges from 40-60% of their total dry mass (Beers 1966; Ara 2001; Nishibe & Ikeda 2007; Ch  telat *et al.* 2012). Assuming this, mean total biomass in 2011 ranged from 3.99 μg DW/L to 5.99 μg DW/L, and maximum summer biomass ranged from 7.74 to 11.61 μg DW/L. This is less than estimates for the time period shortly following dreissenid expansion, when summer total zooplankton biomass was closer to 20 μg DW/L (Barbiero *et al.* 2012; Vanderploeg *et al.* 2012). The 2011 zooplankton biomass numbers estimated in this study may represent a continuation of a trend of decreasing zooplankton in Lake Michigan. However, the majority of earlier zooplankton biomass estimates in Lake Michigan are made at deepwater sites with depths exceeding 100m. Variation in zooplankton concentration can exist between sites, with concentration increasing at deep sites compared to ones at mid-depth sites (Agy 2001). Therefore, because the 2011 biomass estimates were made in water with a depth between 50m and 70m, the estimates may better represent biomass at mid-depths sites in Lake Michigan. More recent full lake averages of zooplankton biomass are needed to determine if this is the case.

The high proportion of copepods to total biomass observed in 2011 is consistent with other studies and is expected for oligotrophic conditions (Barbiero *et al.* 2009, 2012; Vanderploeg *et al.* 2012). Likewise, a low contribution by herbivorous cladocerans was also expected. However, unlike other studies that have noted a large decrease in cyclopoid biomass, *D. thomasi* contributed an appreciable amount to the total zooplankton biomass. Not only was their relative contribution higher compared to other studies, their mean biomass was also much higher than recent estimates (Vanderploeg *et al.* 2012). However, the 2011 biomass estimates were still below pre-Dreissenid expansion population means (Makarewicz *et al.* 1995). These results show that cyclopoids have not completely disappeared from Lake Michigan and are maintaining significant populations, at least in southwestern Lake Michigan.

Average seston biomass estimates in July 2011 were about two times greater than other recent estimates of phytoplankton biomass in Lake Michigan during the mid-stratification period (Fahnenstiel *et al.* 2010). However, these estimates were made by calculating the volume of a phytoplankton cell, converting this to units of carbon, and multiplying by cell concentration in the water column (Fahnenstiel *et al.* 2010). Therefore, differences in techniques could lead to discrepancies between results. Furthermore, because the 2011 seston samples did not exclusively contain phytoplankton and because microplankton contributed little at this time, the additional biomass could be due to the presence of ciliates, heterotrophic nanoflagellates, and bacteria. This is plausible for picoplankton because bacteria can reach considerable biomass in Lake Michigan (Gardner 2004). Likewise, both heterotrophic nanoflagellates and ciliates can contribute substantially to total planktonic biomass with means upwards of 20 μ gC/L (Carrick 2005). Finally, these other recent biomass estimates were made at a single site in southeastern Lake Michigan. Longitudinal transects in Lake Michigan have indicated that chlorophyll concentrations in southwestern Lake Michigan are generally higher than in southeastern Lake

Michigan (Bootsma unpublished). This spatial variation in chlorophyll concentrations could explain the higher seston biomass observed in July 2011 compared to these previous estimates.

Seston biomass peaked on 8/30/2011. This peak was largely due to a considerable increase in microplankton biomass, although the maximum picoplankton biomass was also observed at this time. Though phytoplankton can fall into the microplankton size category, the rotifer *Keratella* was visually abundant in the zooplankton samples during this sampling period. Likewise, the timing of this peak in microplankton corresponds with dreissenid mussel reproduction. In both early summer and early to late fall, dreissenid mussels release a large number of veligers into the water column (Nalepa & Fanslow 2010). Dreissenid veligers can range in size from 50µm to 400µm, falling largely into the size range of the microplankton size class (Ackerman *et al.* 1994). Although nearshore mussels are thought to be responsible for the fall reproduction event, high concentrations have been observed in the pelagic zone during this time (Nalepa & Fanslow 2010). Therefore, it is possible that the peak in microplankton biomass can at least partially be attributed to mussel veligers. If these veligers are being exploited, this large input of biomass into the pelagic zone could provide energy to higher trophic positions at this time.

A few Lake Michigan zooplankton species have been found to be able to feed on dreissenid mussel veligers. In a laboratory study, the calanoid copepods *L. macrurus*, *Leptodiaptomus sicilis*, and *E. lacustris* were given various life stages of dreissenid veligers. Of the three copepods, *E. lacustris* was the only one able to feed efficiently on the veligers (Liebig & Vanderploeg 1995). Furthermore, although *Bythotrephes* aptitude to feed on dreissenid mussel veligers has not been evaluated, the closely related predatory cladoceran *Cercopagis pengoi* can effectively consume dreissenid mussel veligers (Pichlova-Ptacnikova & Vanderploeg 2009). Interestingly, the biomass of both *Bythotrephes* and *E. lacustris* has been observed to have increased modestly following the offshore expansion of dreissenid mussels. The ability of *E.*

lacustris to feed on mussel veligers has been suggested as being directly related to this biomass increase(Vanderploeg *et al.* 2012). Additionally, both *Bythotrephes* and *E. lacustris* peak in biomass and reach their adult life stages during fall(Vanderploeg *et al.* 2012). This temporally overlaps with the nearshore dreissenid mussel reproduction event potentially increasing the exploitation of veligers by these zooplankton species(Nalepa & Fanslow 2010).

2.4.2 Stable Isotope Signatures of Seston Size Classes

No significant difference was found between the $\delta^{15}\text{N}$ signatures of the three seston size classes. This is surprising as there is potential for several trophic transfers in organisms smaller than 210 μm (Weisse 1990) Yet, if a large degree of trophic transfers were taking place between these seston size classes a significant difference between $\delta^{15}\text{N}$ signatures would be expected. Instead the similar $\delta^{15}\text{N}$ signatures observed may suggest that all three size classes are dominated by autotrophic phytoplankton or bacteria. If this were the case and all three size classes were taking up nitrogen from a similar pool no difference in the stable isotope signature between size classes would be expected.

Alternatively, variation in the species composition within size classes between sampling periods may make dissemination of trends difficult trends. Size alone does not determine feeding behavior. Both heterotrophic and autotrophic organisms can exist in all three size classes (Carrick 2005). Any seasonal variation in the species composition within these three size classes should have an effect on the overall $\delta^{15}\text{N}$ signature of the sample. For example, a nanoplankton sample that has more heterotrophic ciliates would be expected to have a higher $\delta^{15}\text{N}$ signature than one dominated by phytoplankton. Heterotrophic nanoflagellates and ciliates biomass has been observed to be about one third of that of phytoplankton in Lake Michigan(Carrick 2005). These protozoans could therefore be potentially contributing to the stable isotope signature of seston.

Because no information was taken on the species composition of the three seston size classes, it is impossible to know how this may have affected the samples.

The $\delta^{13}\text{C}$ of nanoplankton was significantly greater than both microplankton and picoplankton. During trophic transfer, the $\delta^{13}\text{C}$ stable isotope is assumed to be fractionated much less than the nitrogen stable isotope (DeNiro & Epstein 1978; Minagawa & Wada 1984; Vander Zanden & Rasmussen 2001; Post 2002). Because of this, consumers generally have similar $\delta^{13}\text{C}$ signatures to their food sources. This would make the species composition of the sample less of an issue as autotrophs and heterotrophs should have relatively similar $\delta^{13}\text{C}$, providing there is a direct trophic link between the autotrophs and heterotrophs. A couple mechanisms could be driving the difference in the $\delta^{13}\text{C}$ of nanoplankton compared to the other seston samples. In phytoplankton, the stable carbon isotope signature can depend on the source of the dissolved inorganic carbon that they are taking up. Variation can exist between the $\delta^{13}\text{C}$ signatures of major DIC pools with CO_2 generally being less enriched than bicarbonate (Mook, Bommerson & Staverman 1974). Because some phytoplankton species are more efficient at utilizing bicarbonate for photosynthesis, differential enrichment in the $\delta^{13}\text{C}$ signatures between species can exist (Burns & Beardall 1987; Zohary *et al.* 1994). If nanoplankton are more efficiently using bicarbonate as a carbon source it would be expected that they would have a more enriched $\delta^{13}\text{C}$ signature.

A second mechanism that could be leading to the enrichment in the $\delta^{13}\text{C}$ of nanoplankton could be variation in fractionation factors between the seston size classes. This would occur even if all three size classes are taking up carbon from the same pool. Differences in carbon stable isotope fractionation factors have found to be related to phytoplankton species, temperature, growth rates, and geometry of the cell (Wong & Sackett 1978; Laws *et al.* 1995; Popp *et al.* 1998). Any of these parameters could potentially be leading to an increased fractionation factor for nanoplankton resulting in an enriched $\delta^{13}\text{C}$ relative to the other seston size classes. A more intensive study focused on seston carbon stable isotopes would be required to determine what

mechanisms are resulting in the enriched carbon stable isotope the nanoplankton size class in Lake Michigan.

2.4.3 Trophic Position of Zooplankton Species

In food web studies, small organisms like zooplankton are commonly subject to trophic lumping. In this situation morphologically similar species are lumped into a single trophic position (Polis 1991). In the most extreme cases, a simple phytoplankton to zooplankton to fish model is used. Unfortunately, this type of model is not uncommon, despite the wide range of feeding behaviors described for zooplankton species. For example, a lower trophic level food web has been developed for Lake Michigan's plankton community. In this model, zooplankton are lumped into groups of either large zooplankton or small zooplankton (Chen 2004). Large zooplankton includes all copepod species, while small zooplankton encompasses ciliates and heteroflagellates. However, based on the stable isotope results presented here, this appears to be an oversimplification of the system. The N isotope results reveal a maximum trophic position within Lake Michigan's zooplankton community of 4.24. The stable isotope biplot suggests that plankton do fit into discrete trophic categories that are separated by approximately 3.4‰, but these categories span several trophic levels. The simplest interpretation of the trophic estimation data alone would be a linear food web in which *D. mendotae* and *B. longirostris* feed on phytoplankton, most copepods and *Bythotrephes* feed on these cladocerans, and *L. macrurus* feed primarily on copepods species.

However, when zooplankton biomass and trophic position are plotted, an inverted pyramid is produced with primary consumers having substantially less biomass than higher trophic positions. This would suggest two situations. Firstly, inverted biomass pyramids, though rare, have been observed for plankton in both marine and freshwater systems (Gasol, Giorgio &

Duarte 1997; Giorgio *et al.* 1999; Moustaka-Gouni *et al.* 2006). Although the standing biomass of the food source is less than the consumer, the system remains stable because the high productivity of the food source allows them to continuously replenish their population (Morin 1999).

However, this relationship is more commonly observed for herbivorous zooplankton feeding on phytoplankton rather than for species of higher trophic positions (Gasol *et al.* 1997; Giorgio *et al.* 1999; Moustaka-Gouni *et al.* 2006). Furthermore, the two species contributing the most to the secondary consumer biomass, *Leptodiaptomus* and *D. thomasi*, have been typically found to feed little on herbivorous cladocerans. Therefore, it seems more likely that these organisms are feeding on some component of the planktonic food web with a $\delta^{15}\text{N}$ similar to that of *D. mendotae*.

Leptodiaptomus species were not separated for analysis; however, the three species most common in Lake Michigan have similar described feeding ecologies, feeding as omnivores on protozoa and phytoplankton (Vanderploeg *et al.* 2012). The mean trophic position for *Leptodiaptomus* was 3.15, over a full trophic position higher than the assumed primary consumer *D. mendotae*. As mentioned in section 2.2, I was not able to find any significant difference between the $\delta^{15}\text{N}$ signatures of the three seston size classes. However, although not significantly different from the other two size classes of seston, the mean $\delta^{15}\text{N}$ signature of nanoplankton was high relative to the other seston groups and similar to that of *D. mendotae*. If *Leptodiaptomus* were feeding more regularly than *D. mendotae* on some component of the seston community that has a high nitrogen stable isotope signature, its stable isotope signature could be enriched, confounding trophic estimation.

It is well documented that the feeding of calanoid copepods can be highly selective (Strickler & Koehl 1981; DeMott 1988; Wotton 1994). The selectivity of diaptomid species has been very well studied, and it has been found that these calanoids will actively feed on larger particles while rejecting low quality food like cyanobacteria (Vanderploeg, Paffenhofer & Liebig 1988; Wotton 1994). Alternatively, *Daphnid* species have been described as single mode feeders.

This feeding mechanism is more of a generalist feeding style in which all food is retained as long as it is larger than the intersetule space between their filter combs and smaller than what can be processed by the feeding apparatus (Wotton 1994). Therefore, the trophic position of *Leptodiaptomus* could be higher than *D. mendotae* if the particles that *Leptodiaptomus* are selecting are consistently more ^{15}N -enriched than bulk seston caught within *D. mendotae* filter combs. In this situation, the inverted biomass pyramid (Fig. 9) would be misleading since *Leptodiaptomus* would be feeding on some component of the seston biomass rather than directly on *D. mendotae*. This scenario seems more realistic due to the high biomass of *Leptodiaptomus* compared to that of *D. mendotae*.

Furthermore, other studies have consistently found that the $\delta^{15}\text{N}$ of copepods are higher than cladocerans (Leggett *et al.* 2000; Karlsson *et al.* 2004; Matthews & Mazumder 2005). For example, in a study of fourteen lakes in Sweden, copepods were found to have a higher $\delta^{15}\text{N}$ signature and trophic position than cladoceran species in all lakes sampled (Karlsson *et al.* 2004). While copepod $\delta^{15}\text{N}$ signatures were relatively stable, cladoceran $\delta^{15}\text{N}$ signatures were found to strongly correlate to bacterial production (Karlsson *et al.* 2004). The authors suggested that these low $\delta^{15}\text{N}$ signatures were due to cladoceran species feeding generally on low $\delta^{15}\text{N}$ bacteria, while copepods are likely feeding more heavily on protozoans or high $\delta^{15}\text{N}$ baseline algae (Karlsson *et al.* 2004). A more fine scale analysis using stable isotopes of size classes within the seston community or even species specific sampling of protozoans could help to determine the relationship between zooplankton selective feeding and variations in $\delta^{15}\text{N}$ signatures in organisms closer to the base of the food web.

Like *Leptodiaptomus*, *D. thomasi* has little aptitude for feeding on cladoceran species. *Diacyclops* species have been described as being highly selective raptorial feeders with a food preference for microzooplankton. Feeding experiments in Lake Ontario found that *D. thomasi* had little effect on organisms smaller than 20 μm while having a significant effect on large

(~40µm) soft bodied mixotrophic dinoflagellates, large protozoans, and rotifer eggs (Leblanc, Taylor & Johannsson 1997). This size selectivity for *D. thomasi* would result in them feeding more on organisms within the seston size class of nanoplankton.

Other food preference studies have found that *D. thomasi* will consistently select soft bodied rotifers including large *Polyarthra* spp. and *Synchaeta* while avoiding species that possess loricates and spines like *Keratella* and *Bosmina* (Stemberger 1985). Though their abundance has declined in the last decade, both *Synchaeta* and *Polyarthra* make a notable contribution to the total rotifer abundance in Lake Michigan (Barbiero & Warren 2011). Furthermore, *Synchaeta* concentrations have been negatively correlated with *D. thomasi* concentrations in Lake Michigan and both species follow a similar seasonal succession, peaking in spring and early summer (Stemberger & Evans 1984). Therefore, *Synchaeta* may contribute to the diet of *D. thomasi* in Lake Michigan; however, because rotifers were not exclusively sampled, their $\delta^{15}\text{N}$ relative to *D. Thomas* is unknown. Furthermore, because *Synchaeta* are commonly larger than 210µm, they would not have been included in any of the seston samples (Stemberger 1985). Exclusive sampling of rotifers for stable isotope analysis would help to determine their contribution the diet of *D. thomasi* and other Lake Michigan zooplankton species.

Contrary to *D. thomasi* and *Leptodiatomus*, *E. lacustris* has been shown to be able to feed on both large and small cladocerans including *Daphnia*, *Holopedium*, and *Bosmina* (Wong & Sprules 1985; Schulze & Folt 1990). Gut content analysis from organisms taken from Gull Lake Ontario suggested omnivorous feeding patterns with both plant and animal material consistently found in their gut (Wong & Chow-Fraser 1985). Within the laboratory, they can survive and reproduce when fed either plant or animal material exclusively (Schulze & Folt 1990). However, within the field, *E. lacustris* egg abundance has been found to have a significant relationship with both *Holopedium* and *Polyarthra* concentrations, suggesting a relationship between fecundity and carnivory (Schulze & Folt 1990). Within Lake Michigan, *E. lacustris* is

the only copepod known to efficiently feed on Dreissenid mussel veligers (Liebig & Vanderploeg 1995). A modest increase in biomass of *E. lacustris* was observed following the offshore expansion of Dreissenid mussels (Vanderploeg *et al.* 2012). It has been hypothesized that this biomass increase is directly related to its ability to feed on mussel veligers (Vanderploeg *et al.* 2012). Though the exact food source for *E. lacustris* is not clear, a trophic position estimate of a secondary consumer is consistent with the omnivorous to predatory life style described for this species.

Bythotrephes occupied a similar trophic position to the aforementioned copepod species. Because it is a Great Lakes invader, the feeding ecology of *Bythotrephes* has been very well studied. Following its invasion into Lake Michigan, cladoceran biomass of all indigenous species dropped substantially, while copepods were relatively unaffected (Lehman & Caceras 1993; Barbiero & Tuchman 2004a). In the present study the mean trophic position was 2.89, approximately one trophic position above *D. mendotae*, while the mean $\delta^{13}\text{C}$ signature of both *Bythotrephes* and *D. mendotae* were very similar, suggesting a possible predator prey relationship. These results are consistent with the strong feeding preference that *Bythotrephes* has for *Daphnia* species (Vanderploeg *et al.* 1993).

Interestingly, the mean $\delta^{15}\text{N}$ signature and trophic position of *Bythotrephes* fell below all species of copepods sampled. Both laboratory and field studies have shown that *Bythotrephes* can and do consume copepods in Lake Michigan. However, the relatively low $\delta^{15}\text{N}$ signature for *Bythotrephes* observed in this study compared to copepods suggest that *Bythotrephes* are not feeding on copepods in any appreciable amount (Schulz & Yurista 1998). Strong escape responses as well as diel migration behavior have been suggested as mechanisms that help mitigate the effects of *Bythotrephes* on copepod populations (Bourdeau, Pangle & Peacor 2011; Pichlova-Ptacnikova & Vanderploeg 2011). The relative significance of these mechanisms is uncertain, as many of the species that have been shown to exhibit diel migration behavior in the

presence of *Bythotrephes* were found in the epilimnion. For example, *L. ashlandi* was found at higher proportions in the epilimnion compared to the other water column layers, while *D. thomasi* and *L. minutus* were found in the epilimnion almost exclusively. Regardless of the mechanism, the low stable isotope signature compared to all copepod species suggests that *Bythotrephes* is not feeding substantially on copepods in Lake Michigan. This is significant since copepods contribute substantially to the total zooplankton biomass of Lake Michigan. Therefore, *Bythotrephes* impact on energy transfer may be limited to the suppression of cladoceran populations while leaving a large proportion of the zooplankton biomass unaffected. This impact is still important since herbivores cladocerans have been traditionally considered to be an important food source for fish in Lake Michigan.

L. macrurus had the highest mean $\delta^{15}\text{N}$ and was nearly a full trophic position above all other copepods sampled. Other results also suggest a high degree of carnivory for this species. Gut content analysis conducted on animals collected from Gull Lake, Ontario found that only 20% of the *L. macrurus* sampled contained phytoplankton while 85% contained zooplankton, suggesting high rates of carnivory (Wong & Chow-Fraser 1985). Feeding experiments have determined that CIV-CVI stages are capable of feeding on adult *L. ashlandi* during the winter in Lake Michigan, though they have a food preference for copepod nauplii (Warren 1985; Bowers and Warren 1977). More recently, molecular gut content analysis on *L. macrurus* from the Baltic Sea indicated almost exclusive carnivory, with phytoplankton only making a substantial contribution to their diet during the spring diatom bloom (Dahlgren *et al.* 2012). Stable isotope analysis conducted on the zooplankton community from Lake Huron presented similar results, with *L. macrurus* a full trophic position above other copepod species (Jackson *et al.* 2013). The authors suggested *L. macrurus* were likely feeding on *Leptodiaptomus* during the summer months (Jackson *et al.* 2013). Likewise, similar stable isotope results were found for the large calanoid *Tropodiaptomus cunningtoni* in both Lake Malawi and Lake Tanganyika. Despite being

previously described as a herbivore, *T. cunningtoni* had one of the highest $\delta^{15}\text{N}$ signatures of all zooplankton sampled in both lakes. Even more, the $\delta^{15}\text{N}$ signature for this calanoid were similar or even higher than the predatory cyclopoid *Mesocyclops aequatorialis aequatorialis* (Sarvala *et al.* 2003; Ngochera & Bootsma 2010). Collectively these results suggest that calanoid copepods may be more carnivores than what has been previously assumed in Lake ecosystems.

2.4.4 Assumptions of Trophic Estimation

Although these trophic estimations are generally consistent with the described literature for each species, some of the patterns observed could also be explained by violations of the assumptions of the traditional trophic estimation method. Trophic estimation using stable isotopes relies on a few assumptions that have not been well tested for lower food web taxa. Violation of any one of these assumptions could have a major effect on the estimate of the trophic position of the organism. For example, it is assumed that at each trophic transfer a 3.4‰ fractionation of the nitrogen isotope occurs. Though this assumption is widely applied, it is also surprisingly not well tested. For example, Vander Zanden and Rasmussen's fractionation factor was calculated by averaging thirty-five different estimates of fractionation factors. These estimates ranged from -0.7‰ to 9.2‰ (Vander Zanden & Rasmussen 2001). Of these estimates, only one was for a marine copepod whose $\delta^{15}\text{N}$ fractionation factor was reported as 5.8‰ (Checkley & Entzeroth 1985).

The isotope-based trophic estimation placed *Leptodiaptomus* a full trophic position above *D. mendotae*. However, if *Leptodiaptomus* has a higher fractionation factor than the assumed 3.4‰ it would result in a misinterpretation of their trophic position. Even if *D. mendotae* and *Leptodiaptomus* were feeding on the same food source, a higher fractionation factor for *Leptodiaptomus* would result in an overestimation of their trophic position when compared to *D.*

mendotae. More laboratory experiments on isotopic fractionation by zooplankton are needed to better interpret stable isotope data.

Another assumption of isotopic trophic estimation is that all zooplankton species are part of a food web with a common base. Zooplankton species have spatial preferences within the water column, but depth-variation of phytoplankton isotope composition is rarely considered. A zooplankton species feeding within a depth range that has a more enriched $\delta^{15}\text{N}$ signature than the range in which *D. mendotae* is feeding would result in an overestimation of their trophic position. No major difference was found in the stable isotope signatures between seston in the depth specific water column sampling in 2012. Although, this analysis was limited to three sampling periods and only three water depths within the water column were sampled. Further sampling is required to determine if this is a possible mechanism confounding trophic estimation.

A final critical assumption is that the consumers are in isotopic equilibrium with their food source (Peterson & Fry 1987; Hesslein *et al.* 1993; MacAvoy *et al.* 2001; Woodland *et al.* 2012). In other words, when a sample is collected, the $\delta^{15}\text{N}$ signature of a consumer is assumed to be:

$$\delta^{15}\text{N}_{\text{consumer}} = \delta^{15}\text{N}_{\text{food}} + \text{Fractionation Factor}$$

However, this assumption can be easily violated if a consumer is sampled soon after it switches to a new food source or if the $\delta^{15}\text{N}$ signature of its food source rapidly changes. If this happens, a lag period exists before an organism's tissue reflects that of its food source (Fry & Arnold 1982; Hesslein *et al.* 1993; MacAvoy *et al.* 2001). During this lag period, the consumer's tissue does not reflect that of its food source, which can lead to a misinterpretation of trophic position of the consumer.

This is the reason for holding a consumer relative to an assumed primary consumer. By doing this, it can be assumed that the primary consumer is integrating any changes at the base of the food web into their tissue, therefore avoiding the problem of consumer-food source disequilibrium. However, this assumption can be violated if the primary consumer's stable isotope signature is changing at a different rate than the consumer whose trophic position is being estimated. Therefore, *a priori* knowledge of the rate of change to baseline shifts in $\delta^{15}\text{N}$ of both organisms is required to validate this assumption. This is very rarely the case for any field study using stable isotopes.

The mean trophic position of *L. macrurus* was estimated to be 4.24, nearly a full trophic position above all other copepod species. However, in late June of 2011, the $\delta^{15}\text{N}$ signature of several zooplankton species, including both *D. mendotae* and *L. macrurus*, were found to be as high or in some cases higher than what would be expected for Lake Michigan planktivorous fish (Turschak 2013). Samples were taken again two weeks later and almost all of these zooplankton stable isotope signatures had dropped by approximately 8‰. The exception was for *L. macrurus*, which still maintained a stable isotope signature higher than or similar to that of Lake Trout in Lake Michigan, although it had dropped by approximately 3‰. This suggests that some mechanism other than trophic enrichment is causing high $\delta^{15}\text{N}$ signatures for *L. macrurus*.

In Lake Michigan, a seasonal cycle in the $\delta^{15}\text{N}$ signature of particle organic matter has been observed with winter maximums as high as 7‰ followed by summer minimums at 0‰. Bulk zooplankton samples have been observed to follow a similar seasonal trend (Dr. Brian Eadie and Dr. Harvey Bootsma unpublished). These trends are likely due to seasonal changes in nitrogen cycling (Leggett *et al.* 2000; Kumar, Sterner & Finlay 2008). However, it is unknown how quickly individual zooplankton species respond to this trend. If *L. macrurus* is responding more slowly than other zooplankton species, it could be maintaining tissue with a high $\delta^{15}\text{N}$ that

it accrued over winter. Therefore the stable isotope signature of *L. macrurus* would be more of a representation of response to biogeochemical $\delta^{15}\text{N}$ cycling and different tissue N turnover times rather than trophic enrichment. Testing this hypothesis is critical in interpreting the $\delta^{15}\text{N}$ signature of *L. macrurus* before making any conclusions about their trophic position in Lake Michigan.

2.4.5 Conclusion

This study represents the first attempt at estimating the trophic position of Lake Michigan's zooplankton community. With a few exceptions, the stable isotope signatures are consistent with the described feeding ecology for the major zooplankton species. However, some interesting questions arise from this data. Testing a few critical assumptions are necessary before any major conclusion can be drawn. Firstly, the most abundant genus of zooplankton in Lake Michigan, *Leptodiaptomus*, was estimated to be a full trophic position above *D. mendotae*. A few hypotheses were suggested as to why this could be. One of the most interesting is that selective feeding on some component of the microzooplankton community with a more enriched $\delta^{15}\text{N}$ signature could lead to a high enrichment in their $\delta^{15}\text{N}$ compared to *D. mendotae*. Nanoplankton had the highest mean signature of all seston size classes sampled and is within the size range of selectivity expected for *Leptodiaptomus* species. There could be significant implications for the transfer of energy within the system if *Leptodiaptomus* are feeding selectively on only a small component of the total seston biomass. Typically studies on primary production assume that the full phytoplankton pool is available for transfer to higher trophic positions. Instead, if *Leptodiaptomus* is feeding exclusively on nanoplankton this would greatly reduce this amount of energy available to be transferred up the food web. This is especially significant because *Leptodiaptomus* represents the largest pool of biomass in the pelagic zooplankton community. A more intensive study of the seston community in Lake Michigan is necessary to better test this hypothesis.

A second interesting finding was the high trophic position of *L. macrurus* in Lake Michigan. Both in 2011 and 2012, *L. macrurus* consistently had a higher $\delta^{15}\text{N}$ signature than all other species samples. Furthermore the mean trophic position for this species two trophic positions above *D. mendotae* and a full trophic position above all other copepods. Again like *Leptodiaptomus*, this could be significant for the system since *L. macrurus* makes up such a large proportion of the total zooplankton biomass in Lake Michigan. As note early on, trophic transfers are inefficient and a large amount of energy is lost between them. If the trophic position for this large calanoid is as high as what was estimated within this study it could reduce the amount of energy available to upper trophic positions. Although, other mechanism could possible explain this high $\delta^{15}\text{N}$ signature. Firstly, a higher than average nitrogen fractionation factor could result in an overestimation of its trophic position. Secondly, variation in baseline stable nitrogen isotope signatures within the food web could confound analysis. Finally, if *L. macrurus* reacts slowly to seasonal oscillations in the $\delta^{15}\text{N}$ signature of seston it could be retaining a enriched tissue that it accrued over the winter.

Chapter 3: *In Situ* Isotopic Turnover Rates of Three Lake Michigan Copepods

3.1 Introduction and Objectives

Changes in the isotopic composition of an organism's tissue are governed by two mechanisms. Firstly, a net increase in tissue with a different stable isotope signature due to growth can change the overall the overall $^{15}\text{N}:$ ^{14}N . The rate at which this occurs depends on the growth rate of the organism. Secondly, anabolism and catabolism of tissue in the absence of net growth can directly change the stable isotope composition of an organism (Hesslein *et al.* 1993; del Rio *et al.* 2009). This process can be defined as tissue turnover. The isotopic turnover rate represents the combined effect of growth and tissue turnover (Hesslein *et al.* 1993).

The stable isotope signature of an organism's tissue is a time integrated representation of its feeding behavior. The length of the time integration depends on the isotopic turnover rate. Tissue from organisms that have slow isotopic turnover rates will represent their diet over an extended period of time, as it takes longer for them to integrate new isotopes into their tissue. Conversely, organisms that grow quickly or have a high tissue turnover rate will quickly integrate isotopes into their tissue, and the stable isotope composition will mirror that of their recent diet.

In nature, large changes in the stable isotope signature of an organism's food source can occur (Woodland *et al.* 2012). When this happens, a time lag will exist before the stable isotope signature of its tissue will reflect that of its diet (Fry & Arnold 1982; Hesslein *et al.* 1993; MacAvoy *et al.* 2001). The length of this time lag depends on the isotopic turnover rate of the organism. During this time lag, the trophic position of a consumer energy can be misinterpreted if the lag is not accounted for.

The $\delta^{15}\text{N}$ signature of seston in lakes can oscillate within a relatively regular period (Kumar *et al.* 2008; Gu 2009; Woodland *et al.* 2012). In this oscillation, maximum values are observed during the winter and minima are observed shortly after stratification. Though the exact mechanisms have not been fully described, evidence suggests that seasonal minima in the $\delta^{15}\text{N}$ signature of seston is controlled by cyanobacteria introducing isotopically lighter atmospheric nitrogen into the system through nitrogen fixation (Gu 2009). Because the composition of phytoplankton in lakes is believed to follow a seasonal succession, a reduction in the $\delta^{15}\text{N}$ signature seston should occur soon after cyanobacteria become dominate in the plankton community (Sommer *et al.* 1986).

Alternatively, reliance of phytoplankton on different nitrogen sources throughout the year could cause seasonal fluctuations in $\delta^{15}\text{N}$. Because the lighter stable isotope is favored in chemical reactions, and nitrate is the end product of nitrification, it follows that nitrate should have a lighter stable isotope signature than ammonia. Because ammonia is favored by phytoplankton, they will preferentially uptake this isotopically heavier molecule when available (Wetzel 2001). However, during periods of high productivity, ammonium can become depleted, and phytoplankton instead rely on the isotopically lighter nitrate molecule. Therefore, during the productive summer months, a decrease in the stable isotope signature of seston would be expected.

Regardless of the mechanism, these seasonal oscillations in the $\delta^{15}\text{N}$ signature of seston can be reflected in the stable isotope signature of higher trophic level organisms. Though larger organisms like fish can be affected, zooplankton have been observed to be highly sensitive to these seasonal trends (Perga & Gerdeaux 2005). These rapid transitional periods in the $\delta^{15}\text{N}$ signature of seston can complicate trophic estimation of zooplankton species as a time lag will delay zooplankton tissue equilibration with seston. A long time lag between seston and

zooplankton stable isotope signatures could result in a misinterpretation of zooplankton trophic position during period when seston isotopic composition changes significantly.

In chapter 2, it was hypothesized that the relatively high $\delta^{15}\text{N}$ signatures of *L. macrurus* are the result of maintaining tissue that it accrued over the winter period when the seston $\delta^{15}\text{N}$ signature was high. For this to be true, *L. macrurus* would have to respond slower than other zooplankton species to the seasonal oscillation of $\delta^{15}\text{N}$ in seston and, due to a slower growth rate and/or slower tissue turnover time. The objective of this chapter is to determine whether there are differences between *L. macrurus* and other zooplankton species in the time scales of isotopic response to diet, and how any differences might affect the interpretation of stable C and N isotopes with regard to food web structure.

3.2 Methods

3.2.1 Sample Collection

Between 1/16/2013 and 4/30/2013, samples were collected from the water intake pipe at the Linwood Water Treatment Facility. This water intake pipe draws water from a depth of 20 m, approximately two kilometers offshore, eight kilometers north of the Milwaukee Harbor (Fig. 11). In order to collect zooplankton samples, a large hole was cut into the side of a small plastic pail and the hole was covered with a 153 μm mesh. A hose with an average flow rate of $35.95 \pm 5.95 \text{ L min}^{-1}$ that was connected directly to the main water intake pipe was weighted and placed in the bottom of the pail and allowed to run for several hours, with the duration of sampling being recorded to the nearest minute. Excess water was removed through the filter on the sampling apparatus and the zooplankton samples were immediately transported in a 4-L bottle back to lab. Water samples for the analysis of seston stable isotopes were collected in 4-L bottles from the same water intake hose. With a few exceptions, samples were taken weekly during this time period.

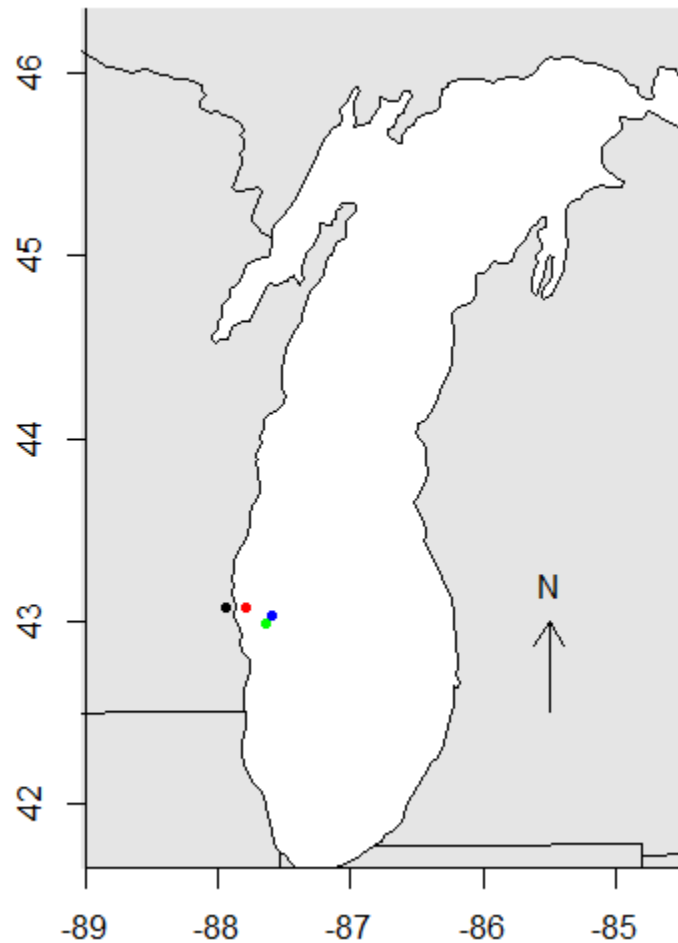


Figure 10: Sample sites for collection of zooplankton and seston samples in 2013. Between 1/16/2013 and 4/29/2012 samples were collected from the Linwood Water Treatment Facility (black dot). Water for these samples were drawn from a site approximately 2 km west of the water treatment facility (red dot). Following this, samples were collected from both a 70m site (blue dot) and a 55m site (green dot).

Because *L. macrurus* has a habitat preference for cold hypolimnetic water during stratification, offshore sampling was necessary following lake stratification in the spring. Due to the increased amount of time required to collect offshore these samples, samples were collected less frequently during this time. On 5/1/2013, samples were collected from the R/V Lake Guardian at the 55m site (42°58'78"N and 87°39'93"W). Zooplankton were collected using a

153 μ m net lowered to approximately one meter from the bottom. Water samples were taken using a rosette sampler that collected water at 10m depth intervals. Approximately equal amounts of water from each sample were combined in a single 4-L bottle to create a pooled sample representing the full water column. Water samples were collected from the same site on 6/19/2013 and 7/16/2013 with the R/V Osprey. Niskin bottles were used to sample from a depth of 2m and then at 10m intervals beginning at 10m. A sample was also taken approximately 1 meter above the lake bottom by attaching a trigger foot to a Niskin bottle, making the bottle close one meter above bottom. These samples were processed individually, and the final stable isotope measurements were average and assumed to represent the full water column.

Zooplankton and seston samples were collected at the 70m site (43° 4' 22.08" N", 87° 47' 24"W) on the following dates in 2013: 5/7, 5/13, 5/22, 5/28, 6/11, 6/17, 7/9, 7/22, 8/6, and 9/11. Zooplankton samples were collected using a 153 μ m net lowered to approximately one meter above the bottom. Seston samples were collected by lowering a Niskin bottle to 10m, 30m, and 50m. Samples were pooled to represent the full water column.

L. macrurus samples and seston were picked from a sediment trap that was moored at the same 55m site. Triplicate polycarbonate tubes with a length of 0.61m and a diameter 0.953 cm were suspended on a mooring cable at depths of 18m, 35m, and 53m. A removable plastic container was attached to the bottom of each tube, and approximately 5 ml of chloroform was added for preservation of the samples. Because *L. macrurus* was most abundant in the 35m sediment traps, they were carefully picked from samples collected at this depth and thoroughly rinsed to remove any sediment or chloroform from the sample. This was done two times, once for a sediment trap deployed between 6/19/2013 and 7/16/2013 and once for a sediment trap deployed between 8/16/2013 and 9/21/2013. The dates midway between deployment and retrieval of the sediment traps were chosen to represent these samples in the time series.

Depth specific seston sampling was also collected from the 55m site. Water samples were collected at 10m intervals between 10 and 50m as well as 2m from the surface and approximately 1m from the lake bottom. On 4/29/2013, the R/V Lake Guardian's rosette sampler was used to collect these samples, while on 6/17/2013 and 7/19/2013, 4-L Niskin bottles were deployed to each depth. All samples were brought back to the lab and immediately processed using the same procedure as mentioned above.

Finally, to determine the timing of stratification and chlorophyll concentrations Seabird Chlorophyll, Temperature, and Depth (CTD) casts were done at both the 55m. The CTD was lowered to approximately one meter above the bottom and then was brought back up again slowly. Casts were done at the 55m site on 4/29/2013, 6/12/2013, 6/19/2013, 7/16/2013, and 8/21/2013.

3.2.2 Sample Processing

Zooplankton and seston samples were processed using the same methodology as described in chapter 2, though seston size classes were not separated. The three copepods that were sampled were *L. macrurus*, *D. thomasi*, and *Leptodiaptomus*. Copepodites and adults were combined for each species. Although an attempt was made to pick *L. ashlandi* exclusively, this was difficult due to the strong similarities of copepodites among the species of this genus and the large number of individuals required for each sample. Therefore, these samples are treated as *Leptodiaptomus spp.* samples rather than a representation of a single species in the genus.

Zooplankton concentration for samples collected at Linwood was calculated by dividing the concentration enumerated in a subsample by the water filtered through the sampling pail. The volume of water filtered was determined by multiplying the flow rate by the filtering time. *In situ* Zooplankton concentration for samples collected offshore was calculated using the same methodology as in chapter 2.

3.2.3 Stable Isotope Analysis

Stable Isotope measurements were made at Washington State University's School of Biological Sciences. The nitrogen and carbon within samples were converted to N_2 and CO_2 gas using an elemental analyzer (ECS 4010 CHNSO Analyzer, Costech Analytical, Valencia CA). The two gasses were separated using a 3m GC column, and the stable isotope ratio of each sample was determined using a continuous flow mass spectrometer (DeltaPlusXP, Thermofinnigan, Bremen). Isotope ratios were held relative to a standard and converted to δ notation using equation 1 (Chapter 1). Vienna Pee Dee belemnite was used as the standard for the stable carbon isotope ($\delta^{13}C = -26.96 \pm 0.04\text{‰}$), while atmospheric nitrogen was used as a standard for the stable nitrogen isotope ($\delta^{15}N = 6.00 \pm 0.11\text{‰}$).

3.2.4 Model Description

A simple model was developed to calculate the $\delta^{15}N$ signature of each of the three copepods as a function of the seston stable isotope ratio, the copepods isotopic turnover rate, and the copepods fractionation factor.

$$NZ_{(t+1)} = \alpha + \beta \quad (\text{Equation 4})$$

In which α is the proportion of old tissue retained during time (t) while β is the proportion of new tissue accrued during time (t). The proportion of old tissue retained (α) can be further broken down into the equation:

$$\alpha = (NZ_{(t)}) (1 - R) \quad (\text{Equation 5})$$

Where R is the isotopic turnover rate at $NZ_{(t)}$ is the stable nitrogen isotope signature at the current time step. The proportion of new tissue accrued (β) can be broken down into the equation:

$$\beta = (R) (NS_{(t)} + Fr) \quad (\text{Equation 6})$$

Where $NS_{(t)}$ is the stable isotope signature of seston at time (t) and Fr is the fractionation factor.

The final equation for the model is therefore:

$$NZ_{(t+1)} = \left((NZ_{(t)}) (1 - R) \right) + \left((R) (NS_{(t)} + Fr) \right) \quad (\text{Equation 7})$$

The model was run at daily time steps. Data gaps for both zooplankton and seston isotope ratios were filled using linear interpolation.

3.2.6 Fractionation Factor

During biochemical reactions, the lighter stable isotope reacts preferentially over the heavy stable isotope (Fry 2006). This results in an increased concentration of the lighter stable isotope in the product of the reaction. The degree of fractionation between the heavy and light stable isotope during reactions or processes is termed the fractionation factor. During trophic transfers, a fractionation factor of 3.4‰ is generally observed (Minagawa & Wada 1984; Vander Zanden & Rasmussen 2001; Post 2002), although it can range from -0.7 to 9.2‰ (Vander Zanden & Rasmussen 2001). Because it was not known if the copepods in this study were explicitly feeding on seston, or if there were intermediary trophic levels between seston and the copepods, it is not valid to assume a 3.4‰ fraction factor. Instead, the fractionation factor was determined by using stable isotope values for periods during which the $\delta^{15}N$ signatures of both seston and zooplankton were stable. During these periods it is assumed that the trophic dynamics were near steady state meaning that zooplankton are in isotopic equilibrium with their food sources. Using this assumption, I calculated the average difference between each copepod and seston during this period at each time step to determine an Effective Fractionation Factor (EFF) in the model.

3.2.8 Isotopic Turnover Rates

The isotopic turnover rate can be defined as the rate of change of an organism's tissue following a change in the stable isotope signature of its food source. The isotopic turnover rate is the sum of both the growth rate and the tissue turnover rate in an organism. The tissue turnover rate is defined as the rate of anabolism and catabolism of copepod protein, assuming anabolism and catabolism are equal (del Rio *et al.* 2009). Isotopic turnover rates was used by fixing the fractionation factor, and iteratively adjusting the tissue turnover rate to obtain the best fit between time series of simulated and empirical measurements of zooplankton $\delta^{15}\text{N}$. Isotopic turnover rates were increased from 0.01 to 0.1 day^{-1} in increments of 0.01 day^{-1} . Following this, isotopic turnover rates were increased from 0.1 to 1 day^{-1} in increments of 0.5 day^{-1} . Increasing the isotopic turnover rate beyond the time step of the model leads to instability. Therefore the maximum isotopic turnover rate of 1 day^{-1} was used.

3.2.7 Growth Rate

Growth rates were calculated to determine the contribution of growth rather than tissue turnover to the overall isotopic turnover rates. The growth rates for individual zooplankton species were determined by first taking the total nitrogen mass of a sample determined through gas chromatography and dividing it by the number of individual plankters on the sample filter. The mean mass of each individual zooplankton was determined for each sample date, and growth rate was estimated by fitting an exponential curve through the data using the equation:

$$W_t = W_i e^{tk} \quad (\text{Equation 8})$$

in which W_t is equal to nitrogen mass at time t , W_i is equal to initial nitrogen mass, and k is the specific growth rate.

3.3 Results

3.3.1 General Results Stable Isotope Results

CTD casts were done at both the 55m and 70m site to determine the timing of stratification and chlorophyll concentrations. Water column temperatures were isothermal on 4/29/2013.

Stratification was first observed on 6/12/2013, with surface temperatures near 12°C and lower water column temperatures near 5.5°C. Temperature differences between the water column layers increased, with epilimnion temperatures near 24°C and 4°C hypolimnion temperatures on 7/16/2013. Stratification remained strong until at least 8/21/2013.

Depth-specific sampling at the 55m site in 2013 showed peak enrichment of $\delta^{15}\text{N}$ near thirty meters on both 6/17/2013 and 7/16/2013 (Fig 8). Likewise, chlorophyll concentrations during both time periods had a maximum value near 30m. Both chlorophyll concentrations and $\delta^{15}\text{N}$ signatures were homogenous throughout the water column on 4/29/2013.

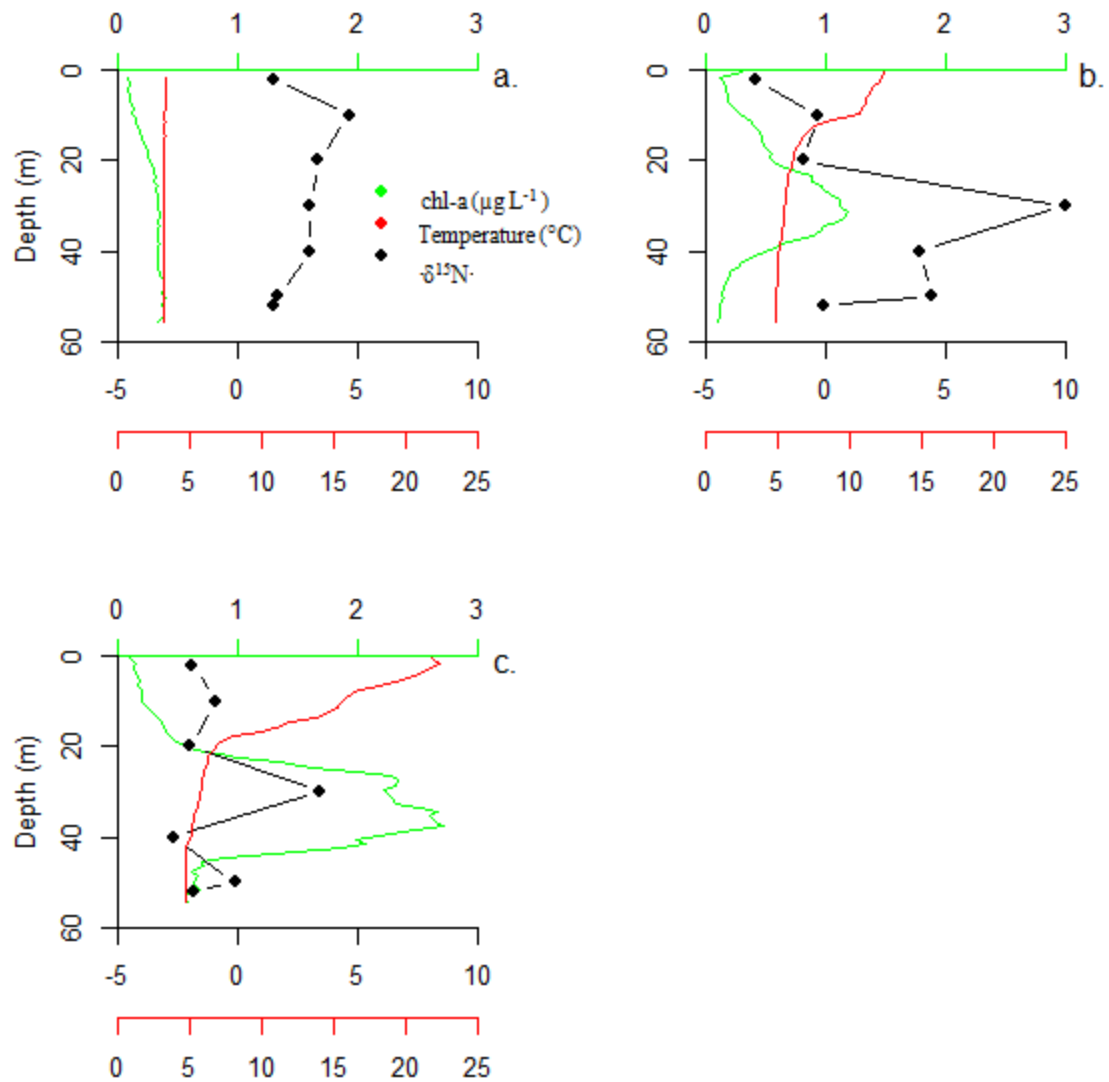


Figure 11: On 4/29/2013 (a.) chlorophyll concentrations, temperature, and sestion stable nitrogen isotope signature were homogenous throughout the water column. On 6/17/2013 (b.) stratification began to set in, a DCM began to form, and the stable nitrogen isotope can be observed increasing with chlorophyll concentrations. This same trend was observed on 7/19/2013 (c.)

During the winter and early spring, the stable isotope signature for all three copepods remained relatively constant, while a very gradual incline of the $\delta^{15}\text{N}$ of sestion during this time period was observed (Fig. 13). Between June and July the stable isotope signatures of both dropped *D. thomasi* and *Leptodiaptomus* from approximately 13 ‰ to 4 ‰ (Fig 16 and 17). The stable isotope signature of these copepods remained low for the rest of the sampling period. Sestion $\delta^{15}\text{N}$ signatures were also

observed to have dropped, reaching a minimum of -1.2 ‰ in mid-July. The stable isotope signature of seston did not remain low and increased near 2.25 ‰ in August and then dropped again to 0.5 ‰ in September. Using equation 2 and assuming that seston occupies a trophic position of 1 the mean trophic position before stratification (1/16/2013 – 6/11/2013) for *Leptodiaptomus* would be 3.56 ± 0.32 and 3.69 ± 0.33 for *D. thomasi*. Following stratification (6/17/2013-9/11/2013) the mean trophic position for *Leptodiaptomus* and *D. thomasi* was 2.42 ± 0.86 and 2.47 ± 0.52 . Finally, A statistically significant correlation was found between the $\delta^{15}\text{N}$ signatures of seston and both *Leptodiaptomus* ($r=0.54, n=19$, p-value: 0.017) and *D.thomasi* ($r=0.76, n= 19$, $p = 0.0002$). chl-a ($\mu\text{g L}^{-1}$)

The $\delta^{15}\text{N}$ signatures of *L. macrurus* did not follow this trend. Instead, the $\delta^{15}\text{N}$ signature of *L. macrurus* stayed relatively consistent throughout the entire sampling period. There was no statistical significant correlation between the $\delta^{15}\text{N}$ signatures of seston and that of *L. macrurus* ($r=0.26, n=19, p=.26$). The mean trophic position of *L. macrurus* before and after stratification was 3.20 ± 0.58 and 3.21 ± 0.51 respectively

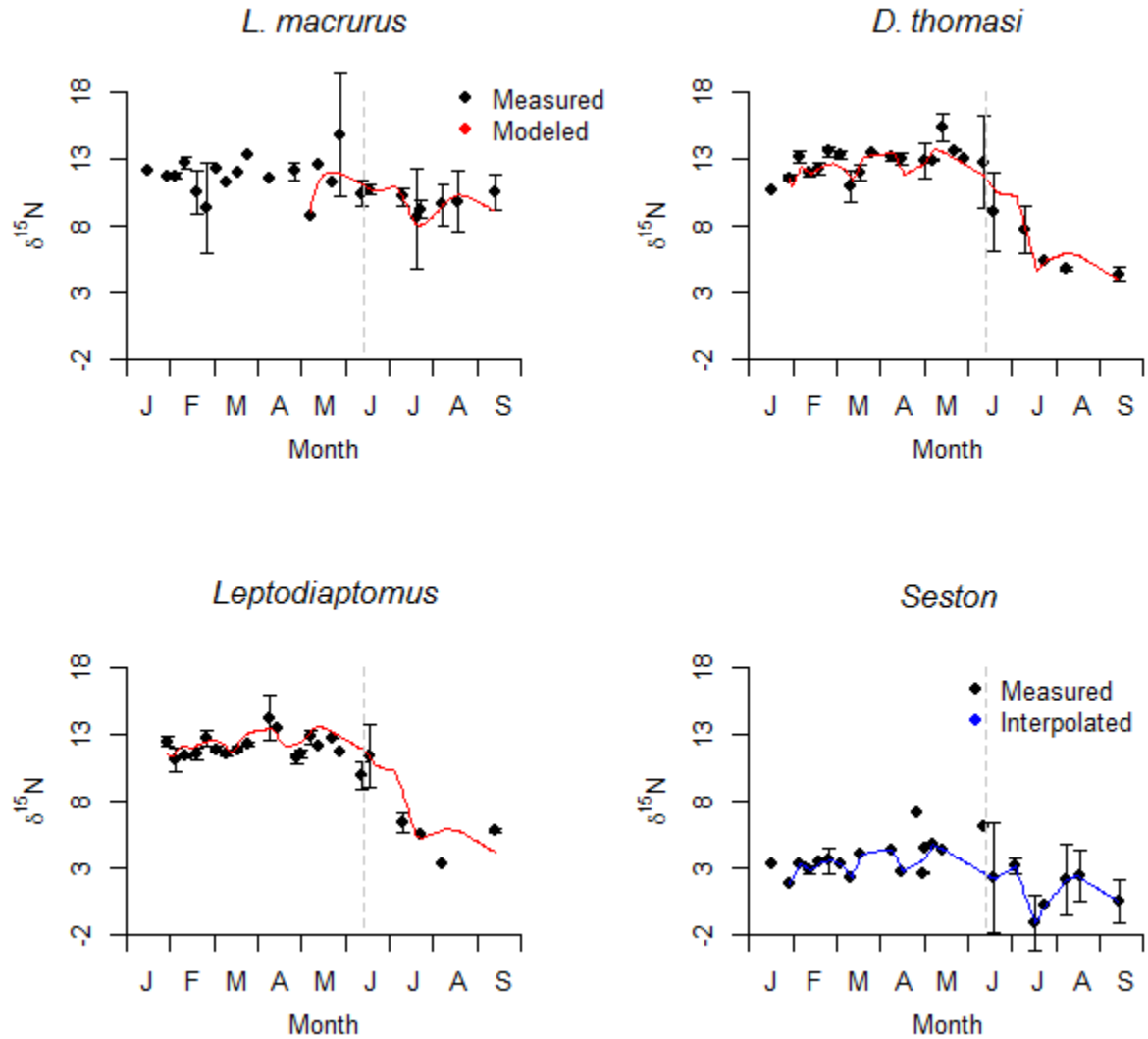


Figure 12: The $\delta^{15}\text{N}$ signatures for three copepods and seston were monitored for 9 months in 2013. Grey lines in the graph signify the approximate start of stratification based on CTD casts done at the 55m site. The $\delta^{15}\text{N}$ signature of *Leptodiaptomus*, *D. thomasi*, and seston drop at the onset of stratification. The $\delta^{15}\text{N}$ signature for these copepods remained low for the rest of the sampling period. *L. macrurus* did not follow this trend, and instead retained a relatively high stable isotope signature throughout the entire sampling period. Two outliers were removed from the seston $\delta^{15}\text{N}$ signatures to when input into the model.

3.3.2 Effective Fractionation Factors

Effective fractionation factors were estimated by taking the difference of the $\delta^{15}\text{N}$ signatures of each zooplankton species and seston for each time step between 1/16/2013 and 5/28/2013 and

averaging these values. These are referred to as effective fractionation factors (EFF), because they do not necessarily represent fractionation due to a single trophic transfer. Rather, they represent the effective discrimination between seston and the zooplankton species. EFF values were similar for all three species during the winter with *D. thomasi*, *L. macrurus*, and *Leptodiaptomus* having EFF's of 9.1, 8.14, and 8.82 respectively. Following the observed drop in the $\delta^{15}\text{N}$ signatures of *Leptodiaptomus* and *D. thomasi* in mid-June, the model was not able to effectively calculate the stable isotope signature of *D. thomasi* and *Leptodiaptomus* using the EFF's calculate over winter. Because of this, an *A priori* alteration of the EFF's for these copepods were used to better model their $\delta^{15}\text{N}$ signatures. The EFF's calculated during winter were used to model the stable nitrogen isotope signatures up until 5/28/2013 (the sample just before the drop in their stable nitrogen isotope signature was observed). Based on the measured data, it appeared that both *Leptodiaptomus* and *D.thomasi* had reached a steady state with their food source by 8/6/2013 and that their stable nitrogen isotope signatures approximately 3.4 ‰ above that of seston. To more effectively model the data a second model was run in which the EFF was not fixed for these copepods. A between 5/28/2013 and 8/6/2013 the EFF of these copepods decreased linearly beginning with an EFF of the winter estimate and ending on 3.4‰. No *A priori* modification was used to calculate the $\delta^{15}\text{N}$ signature of *L. macrurus*.

3.3.3 Isotopic Turnover Rate

For *L. macrurus*, increasing the isotopic turnover rate between 0.01 and 0.15 day⁻¹ quickly resulted in a better fit between the empirical and the modeled $\delta^{15}\text{N}$ signatures. Although, continuing to increase the isotopic turnover rate beyond 0.15 day⁻¹ resulted in a progressively worse fit. Therefore the isotopic turnover rate used to model $\delta^{15}\text{N}$ signatures of *L. macrurus* was 0.15 day⁻¹ ($R^2 = 0.668$). The iterative method provided similar results for both *D. thomasi* and *Leptodiaptomus*. For both copepods, increasing the isotopic turnover rate from 0.01 to 0.15 day⁻¹ greatly increased the fit between the modeled and the empirical data. Unlike for *L. macrurus*,

increasing the isotopic turnover rates for these copepods beyond this value had very little effect on the model fit. For *Leptodiaptomus*, increasing the isotopic turnover rates from 0.15 to 1 day⁻¹ resulted in R² squared values between 0.9288 and 0.9322 with a maximum at an isotopic turnover rate of 0.25 day⁻¹ (R²= 0.9322). Likewise for *D. thomasi*, increasing the isotopic turnover rates from 0.15 to 1 day⁻¹ resulted in R² squared values 0.9413 to 0.9484 with the maximum being observed at 0.45 day⁻¹.

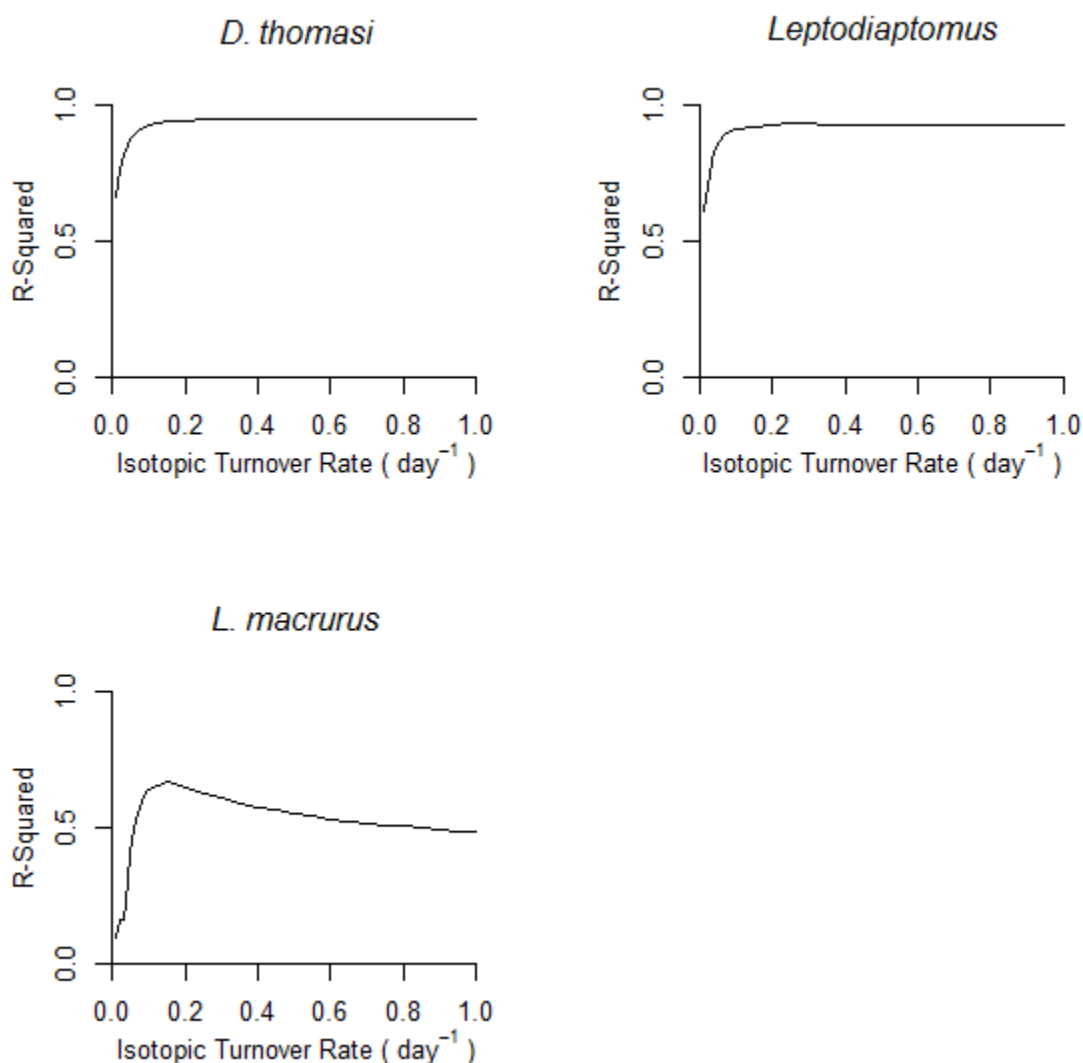


Figure 13: An iterative method was used to determine the best fit between the modeled stable nitrogen isotope signatures with the empirically determined values. Increasing the isotopic turnover rate between 0.15 and 1 day⁻¹ had little effect for *D. thomasi* and *Leptodiaptomus*. Alternatively for *L. macrurus*, the isotopic turnover rate that provided the best fit with the empirical data was 0.15 day⁻¹.

3.3.4 Growth Rates

Growth rates were calculated by fitting an exponential curve to the mean $\mu\text{gN}\cdot\text{individual}^{-1}$ over time (Fig. 14). For *L. macrurus*, during the winter sampling period, total nitrogen individual⁻¹ stayed relatively steady, with a mean value near $2.94 \pm 0.44 \mu\text{g N ind}^{-1}$. During this time period, *L. macrurus* became less abundant within the samples, eventually reaching concentrations too low for stable isotope analysis on 5/1/2013. One week later, the abundance of *L. macrurus* increased. At this time both the length and total mass of each individual were much less than during the winter sampling period. It is likely that the winter samples were composed primarily of the 2012 cohort, while a new 2013 cohort became more prevalent starting in May 2013. Growth rates were calculated for the 2013 cohort. By 7/9/2013 individual *L. macrurus* mass reached values similar to that of their winter average and therefore were assumed to have reached their adult life stage. High growth rates were observed for *L. macrurus* in the spring, with the mass per individual nearly tripling in less than two months. During this period, the growth rate was calculated as 0.0285 day^{-1} ($R^2 = 0.894$). Because the isotopic turnover rate is the sum of growth rate and tissue turnover rate, this growth would contribute 19% to the isotopic turnover rate for *L. macrurus*.

Between 1/16/2013 and 3/18/2013 the mass per individual *D. thomasi* grew steadily increasing from 0.085 to $0.182 \mu\text{g N}^{-1}$. Growth rates for this time were calculated to be 0.0155 day^{-1} ($R^2 = 0.976$). The following week the mass per individual dropped to $0.13 \mu\text{g N}^{-1}$ and then steadily increased to a mass of $0.289 \mu\text{g N}^{-1}$, signifying a new cohort. During this time the growth rate was calculated to be 0.0067 day^{-1} ($R^2 = 0.846$). Again on 8/6/2013 the mass per individual dropped again, possibly indicating the beginning of a third cohort.

The mean mass of nitrogen per individual for the entire sampling period was $0.308 \pm 0.05 \mu\text{g N Individual}^{-1}$. No apparent trend was detectable in the $\mu\text{g N Individual}^{-1}$ for *Leptodiaptomus*. This may be the result of the inability to separate the three major species of

Leptodiaptomus in each sample, as the cohorts of each species may be recruited at different times and grow at different rates. Therefore, it was not possible to quantify growth rate for this species. As a result, the isotope model for *Leptodiaptomus* was adjusted to not include a growth rate, and instead the isotopic composition of this species was modeled solely as a function of isotopic turnover rate and seston isotope composition.

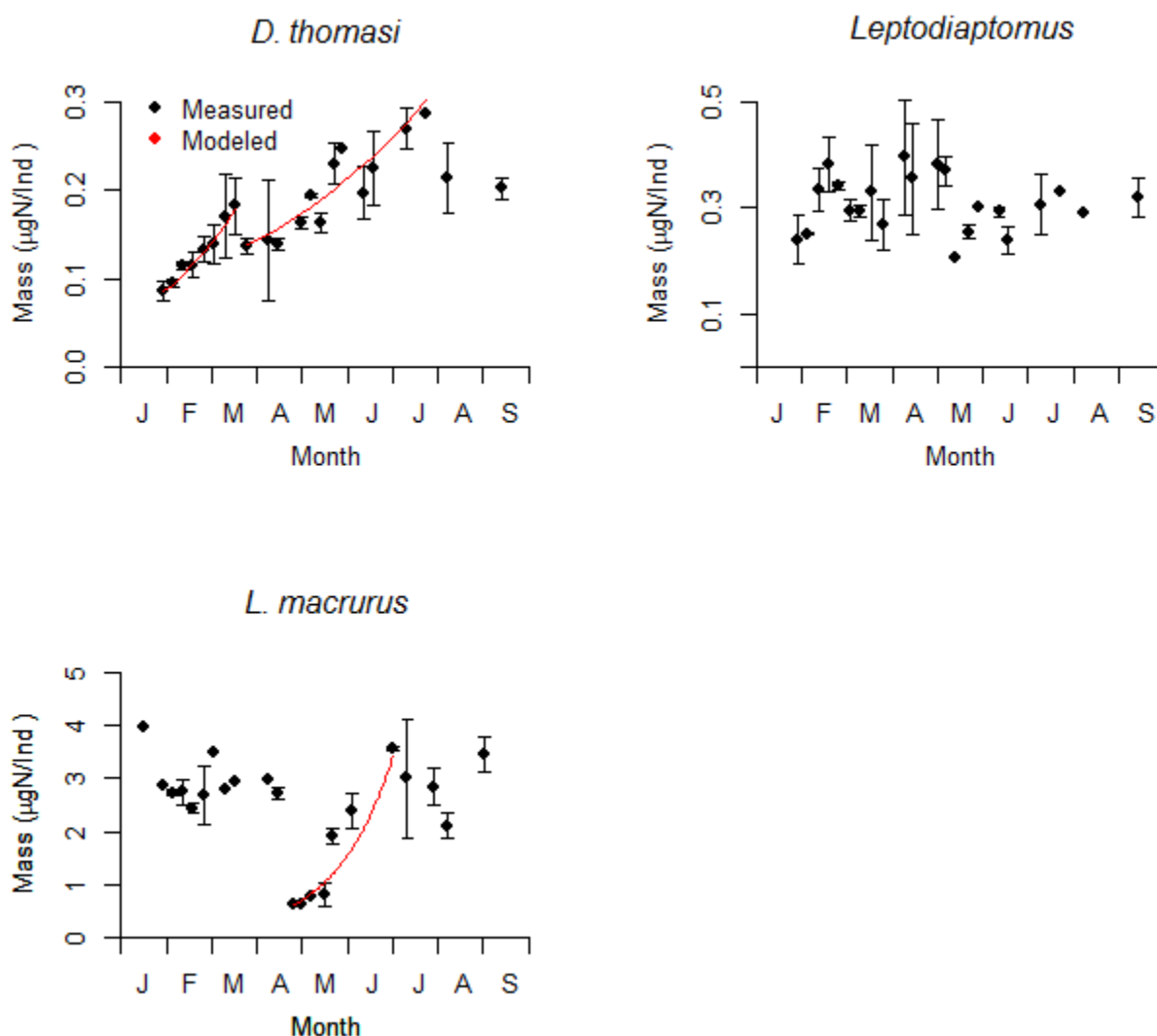


Figure 14: Growth rates were determined calculated by fitting an exponential line to the $\mu\text{gN/ind}$ copepod. Two cohorts can be clearly observed for *D. thomasi*. Over winter, the individual mass of *L. macrurus* remained relatively the same. However, in early April the mass per individual largely decreased. *L. macrurus* reached mass similar to the values observed during winter by mid-June. No apparent trend in growth was observed in *Leptodiaptomus*.

3.4 Discussion

3.4.1 General Discussion

In chapter 2, it was estimated that during the summer of 2011 and 2012 *L. macrurus* was on average more than one full trophic position above both *D. thomasi* and *Leptodiaptomus*. Alternatively, during the winter of 2013 the stable nitrogen signatures of all three of these copepods had similar values. These results suggest that, at least during the winter, these copepods feed at a similar trophic position within Lake Michigan. However, following the onset of stratification the stable nitrogen isotope signature of both *Leptodiaptomus* and *D. thomasi* dropped substantially and remained low for the rest of the summer and into fall. In order to model these observations it was required to reduce the EFF for both of these copepods at this time, suggesting that the $\delta^{15}\text{N}$ signature of the food source for these copepods changed at the onset of stratification. In addition to a reduction in the EFF at this time, a high isotopic turnover rate for these copepods was required to model the empirically determined $\delta^{15}\text{N}$ signatures. Unfortunately, the model had difficulty pinpointing a specific isotopic turnover rate with a range of 0.15 to 1 day^{-1} ; all providing a good fit with the empirical data. The $\delta^{15}\text{N}$ signatures of both *Leptodiaptomus* and *D. thomasi* were significantly correlated to that of seston meaning that the stable nitrogen isotope signature for these copepods tracked seston very tightly. Because of this, it is difficult to identify any time lag between seston and these copepods with the frequency of sampling done within this study. Since the sampling intervals were relatively frequent, this would mean that following a change in the stable nitrogen isotope of their food source, the tissue of these copepods will rapidly reach isotopic equilibrium.

Alternatively, the $\delta^{15}\text{N}$ signature of *L. macrurus* remained relatively consistent throughout the entire sampling period. The model was able to better determine an isotopic turnover rate for *L. macrurus* with a rate of 0.15 day^{-1} providing the best fit with the measured data. If the isotopic turnover rate of an organism's tissue is known it is possible to calculate the

amount of time required for a percentage of its tissue to reach isotopic equilibrium with a new food source using the equation

$$T = -Ln\left(\frac{\alpha}{R}\right)$$

Where T is the time, α is the percentage of tissue, and R is the isotopic turnover rate (del Rio *et al.* 2009). Using this equation it would require 4.62 days for half of their tissue to reach isotopic equilibrium with their food source and 19.97 days for 95% of their tissue to reflect a new food source. This time lag would result in *L. macrurus* maintaining tissue that it accrued over the winter for only a short period of time after the onset of stratification. Therefore, it is unlikely that this is the major cause resulting in the high $\delta^{15}\text{N}$ signature of *L. macrurus* observed during the summer relative to the other copepods. Instead, the difference between the $\delta^{15}\text{N}$ signature of seston and *L. macrurus* changed little between the entire sampling period. In contrast to *D. thomasi* and *Leptodiaptomus*, this would result if the $\delta^{15}\text{N}$ signature of the food source of *L. macrurus* stayed relatively similar throughout the sampling period.

3.4.2 Diet Changes Following Stratification

Historically in Lake Michigan, chlorophyll-a concentrations during the winter were high enough to maintain populations of overwintering copepods. During this period, river discharge and suspension of sediment would cause nearshore waters to become nutrient rich. Following this, water would be transported offshore and subsequently caught in the prevailing counterclockwise gyre that exists in the southern basin. These nutrients would be taken up by phytoplankton which would then form a large doughnut shaped ring of chlorophyll-spanning depths between 40-100m in the southern basin of Lake Michigan. At this time, significantly higher concentrations of both *Leptodiaptomus* and *L. macrurus* were found within this chlorophyll-a doughnut suggesting that it was historically an important food source for these copepods (Kerfoot *et al.* 2008). During 2002

and 2008 this doughnut shaped ring began to diminish(Kerfoot *et al.* 2010a). Chlorophyll-a concentrations decreased significantly while water clarity greatly increased. At the same time dreissenid mussels expanded offshore reaching an estimated average lake wide densities of 8816 individuals/m² by 2005(Nalepa *et al.* 2006). It is hypothesized that dreissenid mussels are directly to blame for this reduction in winter chlorophyll concentrations and the disappearance of this donut. Overwinter adult copepods have been impacted by this with significantly lower concentrations observed in more recent years(Kerfoot *et al.* 2010a).

Additionally, dreissenid mussels can feed on a wide variety of organisms including ciliates and rotifers (J, Loney & Leach 1995; Lavrentyev *et al.* 1995). Although, they have been to select for organisms sized between 5 and 45µm (Sprung & Rose 1988). During the winter period dreissenid mussels may be selectively feeding on phytoplankton and smaller protozoan's while rejecting larger organisms like rotifers and ciliates. Indeed, small zooplankton concentrations have actually increased in coastal waters in late winter(Kerfoot *et al.* 2010b). Therefore, heterotrophic food sources may be more available to copepods during the winter compared to phytoplankton. This could subsequently increase the stable nitrogen isotope signature of copepods if they are relying on these organisms for food. This is consistent with the high $\delta^{15}\text{N}$ signatures relative seston observed during the study for all three copepods over the winter. Likewise, starvation has also been found to increase the $\delta^{15}\text{N}$ signature in zooplankton species(Adams & Sterner 2000). If food is absent enough during the winter to induce starvation the $\delta^{15}\text{N}$ signature of all three copepods would be expected to increase.

Following the onset of stratification the $\delta^{15}\text{N}$ signature of both *Leptodiaptomus* and *D. thomasi* changed rapidly. Within the mass balance model a reduction in the EFF of both species was required to model the empirical data. Alternatively, keeping the EFF for *L. macrurus* fixed provided a better fit with the empirical data. This suggests that the stable isotope signature of the

food source of *Leptodiaptomus* and *D. thomasi* changed after stratification while that of *L. macrurus* remained the same. Two driving mechanisms could be causing these results. Firstly, following stratification the mean trophic position of both *Leptodiaptomus* and *D. thomasi* dropped about 1 trophic position. These means that these copepods may begin to rely more on phytoplankton or some other organisms found lower in the food web at this time. During periods in which the lake is well mixed dreissenid mussels have access to the full water column. This allows them to filter phytoplankton populations more effectively during these periods (Fahnenstiel *et al.* 2010). Alternatively, during stratification the epilimnion is more protected from mussel filtration. This is apparent in that no significant difference has been found in chlorophyll-a concentrations in the epilimnion between pre and post dreissenid mussel offshore expansion (Pothoven & Fahnenstiel 2013). At this time, competition would be reduced for phytoplankton for copepods that have a preference for the epilimnion and dreissenid mussels. Because both *D. thomasi* and *Leptodiaptomus* were found in the epilimnion during stratification a reduced competition from dreissenid mussels at this time may allow them to integrate phytoplankton into their diet more effectively during stratification (Chapter 2). On the other hand, the mean trophic position of *L. macrurus* before and after stratification remained remarkably similar, over two full trophic positions above seston and a full trophic position above the other copepod species during stratification. Based on this, it seems that the feeding behavior of *L. macrurus* varies little throughout the year with this large calanoid feeding at a high trophic position. This is consistent with the carnivores life style described for this species within the primary literature (Chapter 2).

A second driving mechanism for the large difference between the $\delta^{15}\text{N}$ signature of *L. macrurus* and the other copepods as well as seston could be depth specific feeding of these copepods during summer stratification. At the 55m site, it was observed that the $\delta^{15}\text{N}$ signature of seston increased within increasing chlorophyll concentrations. The highest $\delta^{15}\text{N}$

signature for seston was observed within the Deep Chlorophyll Maximum (DCM). Additionally, the DCM was consistently found below the thermocline. As mentioned earlier, both *Leptodiaptomis* and *D. thomasi* are found more consistently within the epilimnion while *L. macrurus* is restricted to colder waters. If *L. macrurus* is feeding more often within the DCM while *Leptodiaptomis* and *D. thomasi* are feeding in the epilimnion the difference in $\delta^{15}\text{N}$ signatures may be due to a difference in the baseline that they are feeding at. For most of the offshore sampling, water was taken at three depths, integrated and assumed to represent the full water column. Because of this the empirical $\delta^{15}\text{N}$ signature of seston is probably a mean value of the three water column layer; lower than the water found in the DCM, but higher than the water found in the epilimnion. If this were the case, all three copepods may have changed their feeding behavior little before and after stratification but instead rapidly changed the composition of their tissue to reflect the baseline of the water column layer that they are feeding within. Therefore, holding these copepods relative to the measured $\delta^{15}\text{N}$ signatures would result in an overestimation of *L. macrurus* trophic position and underestimation of the two other copepods.

3.4.3 Causes of Variation in Isotopic Turnover Rates - Temperature

The mass balance model was not able to provide an exact estimate for the isotopic turnover rates of *Leptodiaptomis* and *D. thomasi*. However the statistically significant correlation between the $\delta^{15}\text{N}$ signatures of both *Leptodiaptomis* and *D. thomasi* and that of seston suggest that the stable nitrogen isotope signatures for these copepods track seston very tightly.. On the other hand, the mass balance model was able to provide an isotopic turnover rate for *L. macrurus* of 0.15 day, indicating that a small time lag exists before the tissue of *L. macrurus* reflects changes in the $\delta^{15}\text{N}$ signature of seston. Although the reasons for the lower isotopic turnover rates for *L. macrurus* are not clear, it has been hypothesized that two of the major mechanisms controlling isotopic turnover rates in organisms are temperature and body size.

Copepods are ectotherms, meaning their body temperature, and hence metabolic rate, is regulated by ambient temperature. Temperature has been found to be one of the most important factors controlling metabolic rates for copepod species (Ikeda *et al.* 2001). As defined in the model, tissue turnover is the rate of anabolism and catabolism of copepod protein in which anabolism and catabolism are equal. Ammonia is the major end product of catabolism of proteins, which is then excreted as nitrogenous waste by copepods. Like other metabolic processes in copepods, nitrogen excretion has been shown to be influenced by temperature. For example, in an analysis of metabolic rates of 43 marine copepods it was found that temperature and body size accounted for 74-80% of the variation in ammonia excretion rates between species (Ikeda *et al.* 2001). It follows that nitrogen stable isotope turnover rates should also increase with increasing temperatures.

Indeed, a growing body of evidence suggests that temperature and isotopic turnover rates have a positive relationship in aquatic organisms. Though many of these studies indicated that growth is the major process regulating isotopic turnover rates, others have found that tissue turnover is the major process leading to changes in isotopic compositions. A relationship between temperature and isotopic turnover rates has been shown experimentally for larval krill, both summer and winter flounder, and to a lesser extent, Arctic sympagic amphipods (Frazer *et al.* 1997; Bosley, Witting & Chambers 2002; Witting *et al.* 2004; Kaufman *et al.* 2008). Additionally, rapid tissue turnover rates have been observed in tropical regions for various taxa, which are attributed largely to high temperatures (McIntyre & Flecker 2006).

Of the copepods sampled, *L. macrurus* is the most temperature sensitive (Balcer, Korda & Dodson 1984). This large calanoid is a glacial relict in Lake Michigan, and it has a circumpolar distribution usually found in cold deep lakes or brackish seas (Balcer *et al.* 1984). Within Lake Michigan, *L. macrurus* has retained a life history consistent with other arctic copepods, reproducing once per year and maintaining large lipid reserves (Vanderploeg *et al.* 1998). It is

considered a cold water stenotherm, with its upper lethal temperature being 18°C (Roff 1973), though it is usually not found in waters with temperatures above 14°C in the nature (Balcer *et al.* 1984). Within the Great Lakes, *L. macrurus* is generally restricted to the hypolimnion but has been observed migrating to the lower metalimnion at night (Balcer *et al.* 1984; Barbiero *et al.* 2005; Bunnell *et al.* 2012; Vanderploeg *et al.* 2012). During the present study, *L. macrurus* exhibited a stronger preference for the hypolimnion than any other zooplankton species sampled (see Chapter 2).

Leptodiaptomus and *D. thomasi* do not have the same temperature restrictions as *L. macrurus*. *D. thomasi* is considered to be a cold water species; however, it tends to concentrate in the upper 20m of the water column or close to the thermocline during stratification (Balcer *et al.* 1984; Barbiero *et al.* 2005; Bunnell *et al.* 2012; Vanderploeg *et al.* 2012). *D. thomasi* has been observed to exhibit diel migration within Lake Michigan, migrating between the epilimnion and metalimnion. However, during the stratified period in 2012, *D. thomasi* was found almost exclusively in the epilimnion. The three major species of *Leptodiaptomus* have different water column depth preferences. *L. minutus* and *L. ashlandi* generally prefer the upper water column, remaining in the epilimnion and metalimnion respectively during stratification (Balcer *et al.* 1984; Barbiero *et al.* 2005; Bunnell *et al.* 2012; Vanderploeg *et al.* 2012). The largest of the three species, *L. sicilis*, prefers the cold hypolimnion water but participates in diel migration in early summer (Balcer *et al.* 1984; Barbiero *et al.* 2005). In 2012, *L. minutus* was found exclusively in the epilimnion during the day and *L. ashlandi* was never found in the hypolimnion. Though *L. sicilis* was found in the hypolimnion, it was also found in the upper water column layers more often than *L. macrurus*. Therefore, during the stratified period of this study, *Leptodiaptomus* and *D. thomasi* most likely spent more time in warmer water than *L. macrurus*. Because temperature is believed to have a significant impact on the tissue (and hence, isotopic) turnover rate of ectotherms, the temperature differences experienced between these copepods during the stratified

period is likely a major factor that explains the observed differences in isotopic turnover rates in this study.

3.4.4 Causes of Variation in Isotopic Turnover Rates – Body Size

Body size is also hypothesized to have a significant effect on tissue and isotopic turnover rates (del Rio *et al.* 2009). It has long been documented by physiologists that the metabolic rates of organisms vary predictably with body size. For example, as far back as 1932 it was shown that the whole body metabolic rate of an organism scales to a power of $\frac{3}{4}$ of the organism's body size (Kleiber 1932). Almost 85 years later, this idea of metabolic scaling has become pervasive within the literature, and when coupled with temperature and stoichiometry, has even been presented as a fundamental theory underlying all ecological processes (Brown *et al.* 2004). Because isotopic turnover rates are controlled by metabolic processes, they should also scale at the same $\frac{3}{4}$ power of body size (del Rio *et al.* 2009). However, only a few studies have attempted to validate this hypothesis. In an analysis of the isotopic turnover rates in bird species, it was found that the isotopic turnover of the carbon stable isotope due to protein turnover alone increased with bird size. Interestingly, the slope of a regression line fit through isotopic turnover rate of the bird species and the bird mass was surprisingly close to the hypothesized $\frac{3}{4}$ scaling (Carleton & Rio 2005). More laboratory work is required to determine if this same scaling applies to aquatic ectotherms and the nitrogen stable isotope.

Although the exact relationship between body size and turnover rates for aquatic ectotherms has not been determined, evidence has been presented that isotopic turnover rates inversely proportional to body size increase with body size. A recent analysis of freshwater invertebrate's stable isotope signatures attempted to find a relationship between variance within time series and the body size of invertebrates (Woodland *et al.* 2012). In this analysis, standard deviation of stable isotope signatures in a time series was assumed to be a function of sensitivity

to seasonal fluctuations at the base of the food web. In other words, an increased variance in a data set would be the result of high sensitivity to changes in stable isotope signatures of POM and, therefore, a high isotopic turnover rate. When zooplankton were included in the analysis, the authors found a weak but significant linear relationship between both the carbon and nitrogen stable isotopes' standard deviation within these time series and the body size of the invertebrates (Woodland *et al.* 2012). Though the exact quantitative relationship between body size and isotopic turnover in freshwater invertebrates is not clear, there is qualitative evidence that the isotopic turnover rate decreases with increasing mass of aquatic invertebrates.

The mass of each individual copepod taxon in this study varied throughout the sampling period. However, individuals from the 2013 cohort of *L. macrurus* contained on average $890 \pm 497\%$ and $659 \pm 380\%$ more $\mu\text{g N}$ than *D. thomasi* and *Leptodiaptomus* respectively. Assuming that nitrogen contributes a similar proportion to the total body weight of each copepod and that scaling of isotopic turnover rates scale with mass with an exponent of $\frac{3}{4}$, it would be expected that *L. macrurus* would have an isotopic turnover rate 5.15 times smaller than *D. thomasi* and 4.11 times smaller than *Leptodiaptomus*. Based on this, I hypothesize that the allometric scaling of isotopic turnover rates is one factor resulting in the smaller isotopic turnover rate observed for *L. macrurus* relative to the other two copepod taxa.

3.4.5 Rapid Turnover Rates in Copepods

The estimated isotopic turnover rates for the copepods estimated in this study are among the highest estimates for any aquatic ectotherm. Because the values determined in this study are based on in situ measurements, they may be less accurate than values determined in controlled laboratory studies. However, similar laboratory derived isotopic turnover rates of this magnitude for aquatic animals have been observed for rapidly growing larval fish (0.22 day^{-1}) and for both adult and juvenile *Daphnia hyaline* (0.133^{-1} and 0.164 day^{-1}) (Grey 2000; Witting *et al.* 2004).

Furthermore, laboratory estimates of isotopic turnover rates of carbon for adult terrestrial pirate bugs, *Orius majusculus*, have been determined to be as high as 0.44 day^{-1} (Madeira *et al.* 2013). Although typical laboratory derived turnover rates for aquatic ectotherms are approximately one to two orders of magnitude smaller than these estimations, almost all of these studies have been for fish species (Hesslein *et al.* 1993; Sakano *et al.* 2005; Suzuki *et al.* 2005; McIntyre & Flecker 2006; Kaufman *et al.* 2008). It is not surprising that fish would have such a smaller isotopic turnover rate compared to copepods due to the considerable differences in body size.

The present study was not the first to show that in natural environments there is a very tight coupling between the stable isotope signatures of seston and that of zooplankton species. For example, rapid turnover of copepod species was observed in Lake Tanganyika (O'Reilly *et al.* 2002). The authors predicted that they would find a linear increase in the $\delta^{15}\text{N}$ signatures from phytoplankton to zooplankton to fish. Instead, the results showed that both phytoplankton and zooplankton had similar $\delta^{15}\text{N}$ signatures and were actually enriched compared to the fish species sampled. They hypothesized that a recent change in the nutrient source with a more enriched $\delta^{15}\text{N}$ signature had entered the system, increasing the signatures of both seston and zooplankton species, but with insufficient duration to be expressed in higher trophic levels. Interestingly, nitrate profiles taken days earlier were suggestive of a deep water upwelling event taking place approximately four to six days prior to the sampling date. Because the deep water in Lake Tanganyika is believed to be ^{15}N -enriched, this may have caused the heavy stable isotope signature observed for seston. Furthermore, because the $\delta^{15}\text{N}$ of the copepods was equal to and not higher than that of the seston, it is likely that they retained some pre-upwelling tissue and were not fully re-equilibrated with the phytoplankton at the time of sampling.

Using the growth independent mass balance model presented here, the isotopic turnover rate required to see the change in the $\delta^{15}\text{N}$ signature of the Lake Tanganyika zooplankton can be estimated. The authors estimated that pre-upwelling zooplankton tissue had a $\delta^{15}\text{N}$ signature near

2.00‰. Assuming a constant seston stable isotope signature of 7.00‰, a fractionation factor of 3.4‰, and modeled with daily time steps over a 5-day period, an isotopic turnover rate of approximately 0.165 day^{-1} would be required for the zooplankton to reach a stable isotope signature of 7‰. This turnover rate is relatively close to the isotopic turnover rate estimated for *L. macrurus* within this study.

Another study has shown rapid changes in the stable isotope signatures of zooplankton in Lake Geneva (western Europe) (Perga & Gerdeaux 2005). A seasonal cycle of plankton $\delta^{15}\text{N}$ similar to that observed in this study was observed with maxima in the winter followed by minima in mid-summer. A study was conducted to determine to what extent lake whitefish respond to these seasonal fluctuations. With a few exceptions, whitefish muscle and liver tissue as well as several zooplankton groups were collected monthly between January 2002 and August 2003. Stable isotope analysis of the samples confirmed that large seasonal fluctuations of isotope ratios in all zooplankton species existed. For bulk copepods, winter maxima of $\delta^{15}\text{N}$ were approximately 11‰ higher than the summer minima. For all but one month of the sampling period, zooplankton made up the major proportion of the whitefish diet as revealed by gut content analysis. Therefore, as expected, the $\delta^{15}\text{N}$ of whitefish muscle and liver tissue exhibited a seasonal variation, though to a smaller extent, with differences between maxima and minima near 2‰ and 5‰ respectively. However, the seasonality of the whitefish tissues was lagged compared to zooplankton. Because splanchnic tissue turns over more quickly than other tissue types the seasonality was reflected in the liver approximately one month later than in the zooplankton species. The time lag was much longer for muscle tissue, and the seasonality lagged behind zooplankton by four to five months.

Overall, these results, as well as those in the present study, demonstrate the large difference between the time scales of stable isotope integration into zooplankton tissue compared to other organisms like fish. This large difference in time integration between these two important

components of aquatic systems makes direct comparison between species complex. In general, stable isotope signatures from fish tissue represent a time integrated value of their diet on the order of a month all the way up to a year (Hesslein *et al.* 1993; Perga & Gerdeaux 2005; Syvaranta, Hamalainen & Jones 2006). Because of this, changes in the stable isotope signature of their food source only affect the composition of their tissue if sustained for a long period of time. Therefore, variations in stable isotope signatures between fish tissue samples are the result of long term differences in what the fish are eating rather than rapid changes in biogeochemical cycles and feeding behavior.

In comparison, stable isotope signatures from copepod tissue seem to represent a time integrated value of their diet on the order of days to weeks. Unlike fish, even a short term change in the stable isotope signature of their food source will quickly be mirrored by the stable isotope composition of their tissue. This leads to the issue of variation in stable isotope signatures of zooplankton caused by biogeochemical cycles masking variations caused by differences in trophic interactions. .

On the other hand, the close coupling between biogeochemical processes and zooplankton stable isotopes could be exploited as a tool to gather information in a unique way. For example, large historical collections of zooplankton samples are commonly kept due to their ease of collection and storage. These collections could be used to construct data sets that reflect long term biogeochemical trends that might not be attained using other methods. The decoupling of biogeochemical trends from trophic trends and the use of zooplankton as biogeochemical indicators could be potential areas of future research.

3.4.6 Intermediate Trophic Transfers

There was a large difference between the $\delta^{15}\text{N}$ signatures of seston and all three copepod species during the winter months. Assuming a 3.4‰ fractionation factor per trophic transfer, several intermediate trophic transfers would exist between seston and the three copepods samples. However, the model calculated the copepods' $\delta^{15}\text{N}$ signature as if they were feeding directly on seston.

Because of this, the isotopic turnover rate estimates would actually be that of the copepods plus the isotopic turnover rate of any intermediate species between copepods and seston. Because all three copepods had similar $\delta^{15}\text{N}$ signatures, they would not be feeding on each other. Likewise, because these three copepods made up the bulk of the zooplankton samples collected at both Linwood and the offshore sites, the size of any intermediate organisms are probably smaller than the mesh size of the nets (153 μm). This size class could include but is not limited to phytoplankton, heterotrophic nanoflagellates, ciliates, and rotifers. Because all of these organisms have high growth rates and population turnover times, the amount of time for their tissue to reflect the isotopic composition of their food source is likely very short (Carrick 2005). Therefore, most of the isotopic turnover rate estimated by the model can likely be attributed to that of the copepods.

3.4.7 Conclusion

The objective of this chapter was to determine if the high $\delta^{15}\text{N}$ signatures of *L. macrurus* in summer could be the result of maintaining tissue that it accrued over the winter period when seston $\delta^{15}\text{N}$ signature was high. Although the model results suggested that *L. macrurus* had a slower isotopic turnover rate compared to the other two copepod species, it was still relatively high compared to other published values. This high isotopic turnover rate of *L. macrurus* likely is not the driving mechanism for why it has a high stable nitrogen isotope signature and therefore trophic position during the summer months. Instead, this calanoid copepod may simply be feeding at a high trophic position year round. Alternatively, depth specific feeding within the

DCM may also result in a relatively high stable nitrogen isotope signature when compared to zooplankton feeding within the epilimnion.

The model results for all three copepods suggested that they have relatively high isotopic turnover rates compared to other laboratory derived results. This rapid turnover rate will result in their tissue very quickly mirroring the stable isotope signature of their food sources. This makes interpretations of their stable nitrogen isotope signatures significantly easier because, upon sampling, there is a high probability that they are close to isotopic equilibrium with their food source. Despite this, there still seems to be some unresolved mechanism affecting the magnitude of their $\delta^{15}\text{N}$ signatures. Over winter, the $\delta^{15}\text{N}$ signatures of all three copepods were very high relative to that of seston. In chapter 2, I presented a few hypotheses as to why *Leptodiaptomus* would have such a high trophic position relative to *D. mendotae*. First, preferential feeding on a water column depth with a high $\delta^{15}\text{N}$ signature could result in substantial enrichment. However, during the winter, the water column is assumed to be well mixed, so vertical variation would not be expected. Second, a fractionation factor higher than the assumed 3.4‰, value for copepod species could also lead to these results. Likewise, high C:N ratios of their food source could also lead to increased fractionation factors (Adams & Sterner 2000). Without more laboratory studies, this is difficult to confirm one way or the other. Finally, selective feeding on a component of the microbial community with an enriched $\delta^{15}\text{N}$ signature relative to the total seston biomass could enrich the $\delta^{15}\text{N}$ signature of the copepod. Further investigation into this research question could help to interpret stable isotope analysis of copepod species in food web studies.

Chapter 4: Conclusion

Analysis of the stable isotopes of carbon and nitrogen was used to estimate the trophic structure of the Lake Michigan zooplankton community. There were clear isotopic distinctions among different species. Most copepods and *Bythotrephes* occupied a trophic position of a secondary consumer, one trophic position above *D. mendotae*. High $\delta^{15}\text{N}$ values for *L. macrurus* implied a high trophic position of a tertiary consumer for this species. Following these results collected in 2011 and 2012, it was hypothesized that the high trophic position for *L. macrurus* was the result of retaining tissue that it accrued over the winter. Contrary to this hypothesis, results from the mass balance model suggested instead that this large calanoid has a relatively rapid isotopic turnover rate. This results in a small time lag between changes in the stable isotope signature of their food source and a re-equilibration of their tissue to reflect this change.

Instead, the stable isotope results may indeed indicate that *L. macrurus* is feeding one trophic position above other copepods in Lake Michigan. If this is case, this could have significant consequences for the Lake Michigan food web. Recently, a similar analysis using stable carbon and nitrogen isotopes was done to determine the trophic structure of Lake Michigan's fish community. Surprisingly, the mean isotope signature of *L. macrurus* is very similar to the pelagic planktivorous alewife, burbot, and rainbow smelt (*Turschak et al.* 2013). This may suggests that these planktivorous fish are feeding on a similar food source as *L. macrurus*, putting them in direct competition with this large calanoid copepod. Because *L. macrurus* contributes a large proportion to the total zooplankton biomass, this would exert additional pressure on an already declining prey fish community (*Madenjian et al.* 2012).

Alternate explanations still exist for the high trophic position of *L. macrurus*. *L. macrurus* may be feeding a different on a food source with a more enriched baseline than other zooplankton

species. Specifically, because the seston stable isotope signatures were found to be enriched within the DCM preferential feeding within this water column could produce these results. Furthermore, since the DCM was found consistently within the hypolimnion this cold water stenotherm is able to take advantage of this food source. This mechanism warrants further research. Likewise, other novel techniques (e.g. molecular gut content analysis) could be coupled with stable isotope analysis to further our understanding of the role of zooplankton within lake food webs.

Works Cited

- Ackerman J.D., Sim B., Nichols S.J. & Claudi R. (1994) Review of the early life history of zebra mussels (*Dreissena polymorpha*) - Comparisons with Maring Bivalves. *Canadian Journal of Zoology - Revue Canadienne de Zoologie* **72**, 1169–1179.
- Adams T.S. & Sterner R.W. (2000) The effect of dietary nitrogen content on trophic level N-15 enrichment. *Limnology and Oceanography* **45**, 601–607.
- Agy M.A. (2001) *Michigan, Changes in the Nearshore and Offshore Zooplankton Communities of Southeastern Lake*. University of Michigan.
- Ara K. (2001) Length-weight relationships and chemical content of the planktonic copepods in the Cananeia Lagoon estuarine system, Sao Paulo, Brazil. *Journal of Plankton Biology and Ecology* **2**, 121–127.
- Balcer M.D., Korda N.L. & Dodson S.I. (1984) *Zooplankton of the Great Lakes : A Guide to the Identification and Ecology of the Common Crustacean Species*. University of Wisconsin Press, Madison, WI.
- Barbiero R.P., Bunnell D.B., Rockwell D.C. & Tuchman M.L. (2009) Recent increases in the large glacial-relict calanoid *Limnocalanus macrurus* in Lake Michigan. *Journal of Great Lakes Research* **35**, 285–292.
- Barbiero R.P., Lesht B.M. & Warren G.J. (2012) Convergence of trophic state and the lower food web in Lakes Huron, Michigan and Superior. *Journal of Great Lakes Research* **38**, 368–380.
- Barbiero R.P., Schacht L.L., Little R.E. & Tuchman M.L. (2005) *Crustacean zooplankton communities in Lake Michigan*. New Dehli.
- Barbiero R.P. & Tuchman M.L. (2004a) Changes in the crustacean communities of Lakes Michigan, Huron, and Erie following the invasion of the predatory cladoceran *Bythotrephes longimanus*. *Environmental Protection* **61**, 2111–2125.
- Barbiero R.P. & Tuchman M.L. (2004b) Changes in the crustacean communities of Lakes Michigan, Huron, and Erie following the invasion of the predatory cladoceran *Bythotrephes longimanus*. *Canadian Journal of Fisheries and Aquatic Sciences* **61**, 2111–2125.
- Barbiero R.P. & Warren G.J. (2011) Rotifer communities in the Laurentian Great Lakes, 1983–2006 and factors affecting their composition. *Journal of Great Lakes Research* **37**, 528–540.
- Beers J. (1966) Studies on the Chemical Composition of the Major Zooplankton Groups in the Sargasso Sea off Bermuda. *American Society of Limnology and Oceanography* **11**, 520–528.
- Bosley K.L., Witting D.A. & Chambers R.C. (2002) Estimating turnover rates of carbon and nitrogen in recently metamorphosed winter flounder *Pseudopleuronectes americanus* with stable isotopes. *Marine Ecology Progress Series* **236**, 233–240.

- Bourdeau P.E., Pangle K.L. & Peacor S.D. (2011) The invasive predator *Bythotrephes* induces changes in the vertical distribution of native copepods in Lake Michigan. *Biological Invasions* **13**, 2533–2545.
- Brown J.H., Gillooly J.F., Allen A.V.M., West G.P. & Savage eoffrey B. (2004) Toward a metabolic theory of ecology. *Ecology* **85**, 1771–1789.
- Bunnell D.B., Keeler K.M., Puchala E.A., Davis B.M. & Pothoven S.A. (2012) Comparing seasonal dynamics of the Lake Huron zooplankton community between 1983–1984 and 2007 and revisiting the impact of *Bythotrephes* planktivory. *Journal of Great Lakes Research* **38**, 451–462.
- Bunnell D.B., Madenjian, Charles P. Timothy D.J., Kostich J.M., Smith K.R. & Adams J. V. (2013) *Status and Trends of Prey Fish Populations in Lake Michigan, 2012*. Ann Arbor, MI.
- Burns B.D. & Beardall J. (1987) Utilization of inorganic carbon by marine microalgae. *Journal of Experimental Marine Biology and Ecology* **107**, 75–86.
- Burns C.W. & Gilbert J.J. (1993) Predation on ciliates by freshwater calanoid copepods: rates of predation and relative vulnerabilities of prey. *Freshwater Biology* **30**, 377–393.
- Cabana G. & Rasmussen J. (1996) Comparing aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences, U.S.A.* **93**, 10844–10847.
- Carleton S.A. & Rio C. (2005) The effect of cold-induced increased metabolic rate on the rate of ¹³C and ¹⁵N incorporation in house sparrows (*Passer domesticus*). *Oecologia* **144**, 226–232.
- Carpenter S.R., Kitchell J.F. & Hodgson J.R. (1985) Cascading Trophic Interactions and Lake Productivity. *BioScience* **35**, 634–639.
- Carrick H.J. (2005) An Under-appreciated Component of Biodiversity in Plankton Communities: The Role of Protozoa in Lake Michigan (A Case Study). *Hydrobiologia* **551**, 17–32.
- Checkley D.M. & Entzeroth L.C. (1985) Elemental and isotopic fractionation of carbon and nitrogen by marine, planktonic copepods and implications to the marine nitrogen cycle. *Journal of Plankton Research* **7**, 553–568.
- Chen C. (2004) Impacts of suspended sediment on the ecosystem in Lake Michigan: A comparison between the 1998 and 1999 plume events. *Journal of Geophysical Research* **109**, C10S05.
- Ch  tat J., Amyot M. & Cloutier L. (2012) Shifts in elemental composition, methylmercury content and $\delta^{15}\text{N}$ ratio during growth of a High Arctic copepod. *Freshwater Biology* **57**, 1228–1240.

- Dahlgren K., Olsen B.R., Troedsson C. & Bamstedt U. (2012) Seasonal variation in wax ester concentration and gut content in a Baltic Sea copepod [*Limnocalanus macrurus* (Sars 1863)]. *Journal of Plankton Research* **34**, 286–297.
- DeMott W.R. (1988) Discrimination between algae and artificial particles by freshwater and marine copepods. *American Society of Limnology and Oceanography* **33**, 397–408.
- DeNiro M.J. & Epstein S. (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* **42**, 495–506.
- Doubek J.P. & Lehman J.T. (2011) Historical biomass of *Limnocalanus* in Lake Michigan. *Journal of Great Lakes Research* **37**, 159–164.
- Fahnenstiel G., Pothoven S., Vanderploeg H., Klarer D., Nalepa T. & Scavia D. (2010) Recent changes in primary production and phytoplankton in the offshore region of southeastern Lake Michigan. *Journal of Great Lakes Research* **36**, 20–29.
- Frazer T.K., Ross R.M., Quetin L.B. & Montoya J.P. (1997) Turnover of carbon and nitrogen during growth of larval krill, *Euphausia superba* Dana: a stable isotope approach. *Journal of Experimental Marine Biology and Ecology* **212**, 259–275.
- Fry B. (2006) *Stable Isotope Ecology*. Springer Science+Business Media, LLC, New York, NY.
- Fry B. & Arnold C. (1982) Rapid $^{13}\text{C}/^{12}\text{C}$ turnover during growth of brown shrimp (*Penaeus aztecus*). *Oecologia* **54**, 200–204.
- Gardner W.S. (2004) Distribution and dynamics of nitrogen and microbial plankton in southern Lake Michigan during spring transition 1999–2000. *Journal of Geophysical Research* **109**, C03007.
- Gardner W.S., Quigley M.A., Fahnenstiel G.L., D.Scavia W. & Frez . A. (1990) . *Large lakes: ecological structure and function*. Springer, New York.
- Gasol J., Giorgio P. del & Duarte C. (1997) Biomass distribution in marine planktonic communities. *Limnology and Oceanography* **42**.
- Giorgio P. a. Del, Cole J.J., Caraco N.F. & Peters R.H. (1999) Linking Planktonic Biomass and Metabolism to Net Gas Fluxes in Northern Temperate Lakes. *Ecology* **80**, 1422.
- Grey (2000) Trophic fractionation and the effects of diet switch pelagic on the carbon stable isotope “signatures” of pelagic consumers. *Verh. Internat. Verein. Limnol.* **27**, 3187–3191.
- Gu B. (2009) Variations and controls of nitrogen stable isotopes in particulate organic matter of lakes. *Oecologia* **160**, 421–31.
- Gulati R., Perez Martinez C. & Siewertsen K. (1995) Zooplankton as a compound mineralising and synthesizing system: phosphorus excretion. *Hydrobiologia* **315**, 25–37.

- Hesslein R.H., Hallard K.A. & Ramlal P. (1993) Replacement of Sulfur, Carbon, and Nitrogen in Tissue of Growing Broad Whitefish (*Coregonus nasus*) in Response to a Change in Diet Traced by $\delta^{34}\text{S}$, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$. *Canadian Journal of Fisheries and Aquatic Sciences* **50**, 2071–2076.
- Ikeda T., Kanno Y., Ozaki K. & Shinada A. (2001) Metabolic rates of epipelagic marine copepods as a function of body mass and temperature. *Marine Biology* **85**, 587–597.
- J M.H., Lonee C.J. & Leach J.H. (1995) Suppression of microzooplankton by zebra mussels: importance of mussel size. *Freshwater Biology* **34**, 379–387.
- Jackson E.W., Doubek J.P., Schaeffer J.S. & Lehman J.T. (2013) Historical and recent biomass and food web relations of *Limnocalanus* in Lake Huron. *Journal of Great Lakes Research* **39**, 404–408.
- Karlsson J., Jonsson A., Meili M. & Jansson M. (2004) $\delta^{15}\text{N}$ of zooplankton species in subarctic lakes in northern Sweden: effects of diet and trophic fractionation. *Freshwater Biology* **49**, 526–534.
- Kaufman M., Gradinger R., Bluhm B. & O'Brien D. (2008) Using stable isotopes to assess carbon and nitrogen turnover in the Arctic sympagic amphipod *Onisimus litoralis*. *Oecologia* **158**, 11–22.
- Kerfoot W.C., Budd J.W., Green S., Cotner J.B., Biddanda B.A., Schwab D.J., *et al.* (2008) Doughnut in the desert: Late-winter production pulse in southern Lake Michigan. *Limnology and Oceanography* **53**, 589–604.
- Kerfoot W.C., Yousef F., Green S.A., Budd J.W., Schwab D.J. & Vanderploeg H.A. (2010a) Approaching storm: Disappearing winter bloom in Lake Michigan. *Journal of Great Lakes Research* **36**, 30–41.
- Kerfoot W.C., Yousef F., Green S.A., Budd J.W., Schwab D.J. & Vanderploeg H.A. (2010b) Approaching storm: Disappearing winter bloom in Lake Michigan. *Journal of Great Lakes Research* **36**, 30–41.
- Kleiber M. (1932) Body size and metabolism. *Hilgardia* **6**, 315–351.
- Korstad J. (1983) Nutrient Regeneration by Zooplankton in Southern Lake Huron. *Journal of Great Lakes Research* **9**, 374–388.
- Kumar S., Sterner R.W. & Finlay J.C. (2008) Nitrogen and carbon uptake dynamics in Lake Superior. *Journal of Geophysical Research* **113**, G04003.
- Laurentyev P.J., Gardner W.S., Cavaletto J.F. & Beaver J.R. (1995) Effects of the Zebra Mussel (*Dreissena polymorpha* Pallas) on Protozoa and Phytoplankton from Saginaw Bay, Lake Huron. *Journal of Great Lakes Research* **21**, 545–557.

- Laws E.A., Popp B.N., Bidigare R.R., Kennicutt M.C. & Macko S.A. (1995) Dependence of phytoplankton carbon isotopic composition on growth rate and [CO₂]_{aq}: Theoretical considerations and experimental results. *Geochimica et Cosmochimica Acta* **59**, 1131–1138.
- Leblanc J.S., Taylor W.D. & Johannsson O.E. (1997) The Feeding Ecology of the Cyclopoid Copepod *Diatrypa thomasi* in Lake Ontario. *Journal of Great Lakes Research* **23**, 369–381.
- Leggett M.F., Johannsson O., Hesslein R., Dixon D.G., Taylor W.D. & Servos M.R. (2000) Influence of inorganic nitrogen cycling on the $\delta^{15}\text{N}$ of Lake Ontario biota. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 1489–1496.
- Lehman J.T. & Caceras C.E. (1993) Food-Web responded to species invasion by a predatory invertebrate- *Bythotrephes* in Lake Michigan. *Limnology and Oceanography* **38**, 879–891.
- Liebig J.R. & Vanderploeg H.A. (1995) Vulnerability of *Dreissena polymorpha* Larvae to Predation by Great Lakes Calanoid Copepods: the Importance of the Bivalve Shell. *Journal of Great Lakes Research* **21**, 353–358.
- Lindeman R.L. (1942) The trophic dynamics aspect of ecology. *Ecology* **23**, 399–418.
- MacAvoy S.E., Macko S.A. & Arneson L.S. (2005) Growth versus metabolic tissue replacement in mouse tissues determined by stable carbon and nitrogen isotope analysis. *Canadian Journal of Zoology* **83**, 631–641.
- MacAvoy S.E., Macko S.A. & Garman G.C. (2001) Isotopic turnover in aquatic predators: quantifying the exploitation of migratory prey. *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 923–932.
- Madeira F., Di Lascio A., Carlino P., Costantini M.L. & Pons X. (2013) Change in carbon stable isotope ratios of the predatory bug *Orius majusculus* after dietary shifts. *Entomologia Experimentalis et Applicata* **148**, 287–296.
- Madenjian C.P., Bunnell D.B., Desorcie T.J., Chriscinske M.A., Kostich J.M. & Adams J. V. (2012) *Status and Trends of Prey fish populations in Lake Michigan, 2011*. Ann Arbor, MI.
- Makarewicz J.C., Bertram P., Lewis T. & Brown E.H. (1995) A Decade of Predatory Control of Zooplankton Species Composition of Lake Michigan. *Journal of Great Lakes Research* **21**, 620–640.
- Martin J.W. & Cash-Clark C.E. (1995) The external morphology of the onychopod “cladoceran” genus *Bythotrephes* (Crustacea, Branchiopoda, Onychopoda, Cercopagidae), with notes on the morphology and phylogeny of the order Onychopoda. *Zoologica Scripta* **24**, 61–90.
- Matthews B. & Mazumder A. (2003) Compositional and inter-lake variability of zooplankton affect baseline stable isotope signatures. *Limnology and Oceanography* **48**, 1977–1987.

- Matthews B. & Mazumder A. (2005) Temporal variation in body composition (C : N) helps explain seasonal patterns of zooplankton $\delta^{13}\text{C}$. *Freshwater Biology* **50**, 502–515.
- McIntyre P.B. & Flecker A.S. (2006) Rapid turnover of tissue nitrogen of primary consumers in tropical freshwaters. *Oecologia* **148**, 12–21.
- Minagawa M. & Wada E. (1984) Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochimica et Cosmochimica Acta* **48**, 1135–1140.
- Mook W.G., Bommerson J.C. & Staverman W.H. (1974) Carbon isotope fractionation between dissolved bicarbonate and gaseous carbon dioxide. *Earth and Planetary Science Letters* **22**, 169–176.
- Morin P. (1999) *Community Ecology*, 1st edn. BLACKWELL PUBLISHING, Malden.
- Moustaka-Gouni M., Vardaka E., Michaloudi E., Kormas K.A., Tryfon E., Mihalatou H., *et al.* (2006) Plankton food web structure in a eutrophic polymictic lake with a history in toxic cyanobacterial blooms. *Limnology and Oceanography* **51**, 715–727.
- Mulholland P.J., Tank J.L., Sanzone D.M., Wollheim W.M., Peterson B.J., Webster J.R., *et al.* (2000) Food resources of stream macroinvertebrates determined by natural-abundance stable C and N isotopes and a N-15 tracer addition. *JOURNAL OF THE NORTH AMERICAN BENTHOLOGICAL SOCIETY* **19**, 145–157.
- Nalepa T.F. & Fanslow D.L. (2010) Recent changes in density, biomass, recruitment, size structure, and nutritional state of *Dreissena* populations in southern Lake Michigan. *Journal of Great Lakes Research* **36**, 5–19.
- Nalepa T.F., Fanslow D.L., Iii A.J.F., Lang G.A., Eadie B.J. & Quigley M.A. (2006) Continued disappearance of the benthic amphipod *Diporeia* spp. in Lake Michigan: is there evidence for food limitation? *Canadian Journal of Fisheries and Aquatic Sciences* **63**, 872–890.
- Nalepa T.F., Fanslow D.L. & Lang G. a. (2009) Transformation of the offshore benthic community in Lake Michigan: recent shift from the native amphipod *Diporeia* spp. to the invasive mussel *Dreissena rostriformis bugensis*. *Freshwater Biology* **54**, 466–479.
- Nalepa T.F., Schloesser D.W., Pothoven S.A., Hondorp D.W., Fanslow D.L., Tuchman M.L., *et al.* (2001) First Finding of the Amphipod *Echinogammarus ischnus* and the Mussel *Dreissena bugensis* in Lake Michigan. *Journal of Great Lakes Research* **27**, 384–391.
- Ngochera M.J. & Bootsma H.A. (2010) Temporal trends of phytoplankton and zooplankton stable isotope composition in tropical Lake Malawi. *Journal of Great Lakes Research*.
- Nishibe Y. & Ikeda T. (2007) Metabolism and elemental composition of four oncaeid copepods in the western subarctic Pacific. *Marine Biology* **153**, 397–404.

- O'Reilly C.M., Hecky R.E., Cohen A.S. & Plisnier P.D. (2002) Interpreting stable isotopes in food webs: Recognizing the role of time averaging at different trophic levels. *Limnology and Oceanography* **47**, 306–309.
- Paine R.T. (1988) Food Webs - Road Maps of Interactions of Grist for Theoretical Development. *ECOLOGY* **69**, 1648–1654.
- Perga M.E. & Gerdeaux D. (2005) “Are fish what they eat” all year round? *Oecologia* **144**, 598–606.
- Peterson B.J. & Fry B. (1987) Stable Isotopes in Ecosystem Studies. *Annual Review of Ecology and Systematics* **18**, 293–320.
- Pichlova-Ptacnikova R. & Vanderploeg H.A. (2009) The invasive cladoceran *Cercopagis pengoi* is a generalist predator capable of feeding on a variety of prey species of different sizes and escape abilities. *Fundamental and Applied Limnology* **173**, 267–279.
- Pichlova-Ptacnikova R. & Vanderploeg H.A. (2011) The quick and the dead: might differences in escape rates explain the changes in the zooplankton community composition of Lake Michigan after invasion by *Bythotrephes*? *Biological Invasions* **13**, 2595–2604.
- Polis G.A. (1991) Complex Trophic Interactions in Deserts: An Empirical Critique of Food-Web Theory. *The American Naturalist* **138**, 123–155.
- Popp B.N., Laws E.A., Bidigare R.R., Dore J.E., Hanson K.L. & Wakeham S.G. (1998) Effect of Phytoplankton Cell Geometry on Carbon Isotopic Fractionation. *Geochimica et Cosmochimica Acta* **62**, 69–77.
- Post D.M. (2002) Using stable isotopes to estimate trophic position: Models, methods and, assumptions.. *Ecology* **83**, 703–718.
- Pothoven S.A. & Fahnenstiel G.L. (2013) Recent change in summer chlorophyll a dynamics of southeastern Lake Michigan. *Journal of Great Lakes Research* **39**, 287–294.
- Pothoven S.A., Fahnenstiel G.L. & Vanderploeg H.A. (2010) Temporal trends in Mysis relicta abundance, production, and life-history characteristics in southeastern Lake Michigan. *Journal of Great Lakes Research* **36**, 60–64.
- Del Rio C.M., Wolf N., Carleton S. a & Gannes L.Z. (2009) Isotopic ecology ten years after a call for more laboratory experiments. *Biological reviews of the Cambridge Philosophical Society* **84**, 91–111.
- Roff J.C. (1973) Oxygen consumption of *Limnocalanus macrurus* Sars (Calanoida, Copepoda) in relation to environmental conditions. *Canadian Journal of Zoology* **51**, 877–885.
- Sakano H., Fujiwara E., Nohara S. & Ueda H. (2005) Estimation of nitrogen stable isotope turnover rate of *Oncorhynchus nerka*. *Environmental Biology of Fishes* **72**, 13–18.

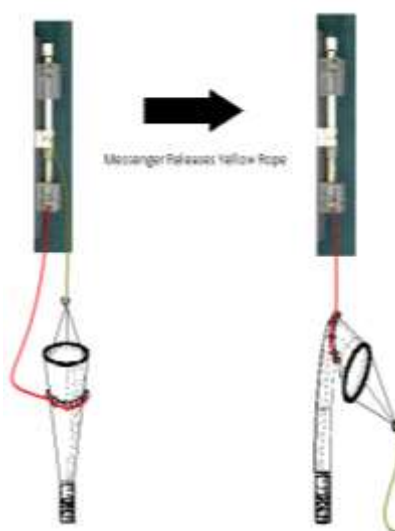
- Sarvala J., Badende S., Chitamwebwa D., Juvonen P., Mwape L., Mölsä H., *et al.* (2003) Size-Fractionated $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ Isotope Ratios Elucidate the Role of the Microbial Food Web in the Pelagial of Lake Tanganyika. *Aquatic Ecosystem Health & Management* **6**, 241–250.
- Schulz K.L. & Yurista P.M. (1998) Implications of an invertebrate predator's (*Bythotrephes cederstroemi*) atypical effects on a pelagic zooplankton community. *Hydrobiologia* **380**, 179–193.
- Schulze P.C. & Folt C.L. (1990) Food Resources, Survivorship, and Reproduction of the Omnivorous Calanoid Copepod *Epischura Lacustris*. *Ecology* **71**, 2224–2240.
- Smyntek P.M., Teece M. a., Schulz K.L. & Thackeray S.J. (2007) A standard protocol for stable isotope analysis of zooplankton in aquatic food web research using mass balance correction models. *Limnology and Oceanography* **52**, 2135–2146.
- Sommer U., Gliwicz Z.M., Lampert W. & Duncan A. (1986) The PEG-model of seasonal succession of planktonic events in fresh waters. *Archiv Fur Hydrobiologie* **106**, 433–471.
- Sprules W.G. & Bowerman J.E. (1988) Omnivory and food chain length in zooplankton food webs. *Ecology* **69**, 418–426.
- Sprung M. & Rose U. (1988) Influence of food size and food quantity on the feeding of the mussel *Dreissena polymorpha*. *Oecologia* **77**, 526–532.
- Stemberger R.S. (1985) Prey selection by the copepod *Diacyclops thomasi*. *Oecologia* **65**, 492–497.
- Stemberger R.S. & Evans M.S. (1984) Rotifer Seasonal Succession and Copepod Predation in Lake Michigan. *Journal of Great Lakes Research* **10**, 417–428.
- Strickler J.R. & Koehl M.A.R. (1981) Copepod feeding currents: Food capture at low Reynolds number. *Limnology and Oceanography* **26**, 1062–1073.
- Suzuki K.W., Kasai A., Nakayama K. & Tanaka M. (2005) Differential isotopic enrichment and half-life among tissues in Japanese temperate bass (*Lateolabrax japonicus*) juveniles: implications for analyzing migration. *Canadian Journal of Fisheries and Aquatic Sciences* **62**, 671–678.
- Syvaranta J., Hamalainen H. & Jones R.I. (2006) Within-lake variability in carbon and nitrogen stable isotope signatures. *Freshwater Biology* **51**, 1090–1102.
- Turschak B. (2013) *Changes in the Lake Michigan Trophic Structure: As Revealed by Stable C and N Isotope*. UW-Milwaukee.

- Vanderploeg H. a., Cavaletto J.F., Liebig J.R. & Gardner W.S. (1998) *Limnocalanus macrurus* (Copepoda: Calanoida) retains a marine arctic lipid and life cycle strategy in Lake Michigan. *Journal of Plankton Research* **20**, 2029–2029.
- Vanderploeg H.A., Liebig J.R., Nalepa T.F., Fahnenstiel G.L. & Pothoven S.A. (2010) *Dreissena* and the disappearance of the spring phytoplankton bloom in Lake Michigan. *Journal of Great Lakes Research* **36**, 50–59.
- Vanderploeg H.A., Liebig J.R. & Omair M. (1993) Bythotrephes predation on Great Lakes zooplankton Measured by an in situ method: Implications for zooplankton community structure. *Archiv Fur Hydrobiologie* **127**, 1–8.
- Vanderploeg H.A., Paffenhofer G.A. & Liebig J.R. (1988) Diaptomus vs. net phytoplankton: effects of algal size and morphology on selectivity of a behaviorally flexible, omnivores copepod. *Bulletien of Marine Science* **43**, 377.
- Vanderploeg H.A., Pothoven S.A., Fahnenstiel G.L., Cavaletto J.F., Liebig J.R., Stow C.A., *et al.* (2012) Seasonal zooplankton dynamics in Lake Michigan: Disentangling impacts of resource limitation, ecosystem engineering, and predation during a critical ecosystem transition. *JOURNAL OF GREAT LAKES RESEARCH* **38**, 336–352.
- Warren G.J. (1985) Predaceous feeding habits of *Limnocalanus macrurus*. *Journal of Plankton Research* **7**, 537–552.
- Weisse T. (1990) Trophic Interaction among heterotrophic microplankton, nanoplankton, and bacteria in Lake Constance. *Hydrobiologia* **191**, 111–122.
- Wetzel R.G. (2001) *Limnology: Lake and River Ecosystems*, 3rd edn. Acedemic Press, San Diego, CA.
- Witting D.A., Chambers R.C., Bosley K.L. & Wainright S.C. (2004) Experimental evaluation of ontogenetic diet transitions in summer flounder (*Paralichthys dentatus*), using stable isotopes as diet tracers. *Canadian Journal of Fisheries and Aquatic Sciences* **61**, 2069–2084.
- Wong C.K. & Chow-Fraser P. (1985) The food of three large freshwater calanoid copepods: *Limnocalanus macrurus* Sars, *Epischura lacustris* Forbes, and *Senecella calanoides*. *Verh. Internat. Verein. Limnol.* **38**, 3190–3194.
- Wong C.K. & Sprules W.G. (1985) Size-Selective Feeding by the Predatory Copepod *Epischura lacustris* Forbes. *Canadian Journal of Fisheries and Aquatic Sciences* **42**, 189–193.
- Wong C.K. & Sprules W.G. (1984) The swimming behavior of the freshwater calanoid copepods *Limnocalanus macrurus* Sars, *Senecella calanoides* Juday and *Epischura lacustris* Forbes. *Journal of Plankton Research* **8**, 79–90.
- Wong W.W. & Sackett W.M. (1978) Fractionation of stable carbon isotopes by marine phytoplankton. *Geochimica et Cosmochimica Acta* **42**, 1809–1815.

- Woodland R.J., Magnan P., Glémet H., Rodríguez M. a & Cabana G. (2012) Variability and directionality of temporal changes in $\delta(13)\text{C}$ and $\delta(15)\text{N}$ of aquatic invertebrate primary consumers. *Oecologia* **169**, 199–209.
- Wotton R.S. (1994) *The Biology of Particles in Aquatic Systems*, 2nd edn. CRC Press, Inc, Boca Raton, Florida.
- Vander Zanden J. & Rasmussen J. (2001) Variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ trophic fractionation : Implications for aquatic food web studies. *American Society of Limnology and Oceanography* **46**, 2061–2066.
- Zohary T., Erez J., Gophen M., Berman-Frank I. & Stiller M. (1994) Seasonality of stable carbon isotopes within the pelagic food web of Lake Kinneret. *Limnology and Oceanography* **39**, 1030–1043.

Appendix A: Construction of a closing zooplankton net

In 2012, a closing zooplankton net was constructed to sample specific water column depths. The closing mechanism from a Niskon bottle was removed, smoothed, and attached to a flat piece of stainless steel. Metal rings were hand sewn around the neck of a 153 μ m mesh net with even distribution. To sample, the closing mechanism was attached near the bottom to the line of a winch. A metal ring was tied to the top of one rope (yellow in the figure) and slid into the release slot on the closing mechanism. The other end of this yellow rope was attached to the top of the net. A second rope (red in the figure) was threaded through the metal rings attached to the net and then secured at the base of the sampling mechanism. The red rope was given enough slack so that it did not inhibit sampling. The full sampling apparatus was lowered to the chosen depth. Sampling then proceeded as normal by bringing the net up through the water column. The depth of the net was monitored using shipboard sonar. At approximately 5 meters before the net was to be closed, a messenger was sent down to trigger the closing mechanism. Once triggered, the yellow rope would be released and the tension would be switched to the red rope, synching the net shut. The net would then be brought up through the water column and retrieved. The full sampling apparatus was tested at 10m while divers were present in the water.



Appendix B: Stable Isotope and Biomass Summary Data

Mean zooplankton biomass and stable isotope signatures.									
Species	TP	$\delta^{15}\text{N}$ (‰)	N	$\delta^{13}\text{C}$ (‰)	n	2011 Biomass ($\mu\text{gC/L}$)	n	2011 Biomass (%)	n
<i>L. macrurus</i>	4.24	10.26 ± 0.94	13	-23.63 ± 0.54	13	0.69 ± 0.25	7	$26.19\% \pm 0.09$	7
<i>D. thomasi</i>	3.38	6.69 ± 0.34	4	-25.26 ± 0.32	4	0.45 ± 0.21	7	$22.85\% \pm 0.10$	7
<i>E. lacustris</i>	3.36	6.21 ± 1.04	4	-24.87 ± 0.6	4	0.03 ± 0.03	7	$2.12\% \pm 0.02$	7
<i>Leptodiaptomus spp.</i>	3.15	6.8 ± 0.8	14	-26.03 ± 0.27	14	1.13 ± 0.34	7	$45.29\% \pm 0.05$	7
<i>Bythotrephes</i>	2.89	5.65 ± 0.68	7	-24.317 ± 0.49	7	0.05 ± 0.03	7	$2.66\% \pm 0.02$	7
<i>B. longirastri</i>	2.52	3.68	1	-25.77 ± 0.05	2	0.02 ± 0.02	7	$0.41\% \pm 0.004$	7
<i>Daphnia</i>	2.00	2.77 ± 0.61	15	-24.14 ± 0.52	15	0.01 ± 0.01	7	$0.48\% \pm 0.002$	7

Appendix C: Seston Stable Isotope Summary Data

Mean seston biomass and stable isotope signatures								
Sample	$\delta^{15}\text{N}$	n	$\delta^{13}\text{C}$	N	Biomass	n	Biomass	n
	(‰)		(‰)		($\mu\text{gC/L}$)		(%)	
Microplankton	1.07 ± 0.8	7	-27.7 ± 0.62	8	35.10 ± 24.02	8	$16.19\% \pm 0.07$	8
Nanoplankton	2.73 ± 0.81	7	-26.00 ± 0.52	7	42.84 ± 6.36	8	$38.28\% \pm 0.06$	8
Picoplankton	0.46 ± 0.65	6	-27.53 ± 0.33	8	54.19 ± 8.55	8	$45.35\% \pm 0.03$	8
Total 2011					131.53 ± 32.37	8		
Epilimnetic*	-0.56 ± 0.10	2	-26.56 ± 0.43	2	129.51 ± 19.15	3	$39.56\% \pm$	3
Metalimnetic*	-1.31 ± 0.52	2	-28.22 ± 1.05	2	142.38 ± 19.18	3	$43.49\% \pm$	3
Hypolimnetic*	-0.19	2	$-28.54 \pm$	2	55.50 ± 5.12	3	$16.95\% \pm$	3

* Indicates 2012 sample. All other samples are from 2011.

Paired Student's t-tests Results for 2011 Seston Size Class stable isotope Signatures		
Seston Size Class	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
Nanoplakton-Microplankton	0.19	0.0081*
Nanoplakton-Picoplankton	0.22	0.018*
Microplankton-Picoplankton	0.94	0.34

* Indicates statistical significance (p-value > 0.05)