

Population dynamics of *Holopedium gibberum* in a Pacific Northwest drinking water reservoir:
effects of temperature, food, and competition

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Abstract

Population dynamics of *Holopedium gibberum* in a Pacific Northwest drinking water reservoir:
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Holopedium gibberum is one of the few cladocerans to create a gelatinous sheath. For this reason it can clog drinking water filtration facilities. In order to better understand the population dynamics of *Holopedium* in a reservoir in the Cascade foothills, *in-situ* single variable treatments were used to investigate the effects of temperature, food, and turbidity. The fatty acid composition of *Holopedium*, *Daphnia* and the seston was tested to examine likely food sources and the possibility of direct competition. It was found that temperature limits the *Holopedium* population during the spring phytoplankton bloom and low food quality limits the population during the late summer. Competitive exploitation between *Holopedium* and *Daphnia* is possible. The composition of several fatty acids in the cladocerans was correlated, indicating shared resources. *Daphnia* followed the seasonal trends in the seston fatty acid composition more closely than *Holopedium*, indicating that the overlap in resources was not complete. *Holopedium* appears to be using the food resources most commonly found above the thermocline and may be able to out-compete *Daphnia* under conditions when chrysophytes or allochthonous fatty acid sources dominate in the seston, however, *Daphnia* appears to utilize a wider range of food resources.

Introduction

Many large Pacific Northwest municipalities, including Seattle, Portland, Vancouver B.C., Everett, and Bellingham obtain their drinking water from reservoirs located in the western slopes of the Cascade Mountains. The source reservoirs are generally oligotrophic to mesotrophic, with little human disturbance in their watersheds, and consistently produce high quality water. The quality of the source water affects the types of water treatment necessary to meet potable water standards. Municipalities such as Seattle and Portland do not use filtration due to their generally low turbidity water and protected watersheds. Other municipalities, such as Everett and Bellingham, have direct filtration plants. Direct filtration differs from conventional filtration in that flocculation is not followed by sedimentation prior to filtration. The direct filtration process is efficient from both economic and operational standpoints. However, direct filtration is more affected by deviations in the source water quality than conventional filtration. The high quality of the source water from the Cascade foothills allows direct filtration plants to be practical.

The City of Everett takes its drinking water from Lake Chaplain Reservoir which is located in the Cascade foothills, seven km north of Sultan, Washington. Its primary inflow is from Spada Reservoir, a larger system located at a higher elevation. Spada Reservoir is operated as a hydroelectric facility by the Snohomish County Public Utility District. The water quality from Spada is consistently high during the summer. However in the fall, when rains re-suspend sediments in the reservoir or erode clay deposits in the watershed, Spada can become turbid. During these times, Lake Chaplain serves an important function as a pre-sedimentation basin for the Everett Water Filtration Plant.

During the summer there are often periods of reduced filter performance at the Everett Filtration Plant. In 2004 the primary source of the intermittent filter clogging was identified as *Holopedium gibberum* (Spotts and Brett 2006). *Holopedium* clog the anthracite coal filters in the plant due to the gelatinous sheath characteristic of this zooplankter (Figure 1). The sheath is larger than the filter pore size, presumably leading to a gelatinous surface mat during periods of high *Holopedium* abundance. During severe episodes, the City of Everett bypasses Lake Chaplain and takes water directly from Spada Reservoir. This is problematic for two reasons, the first being that the operational configuration necessary for the bypass negatively affects the PUD's ability to generate hydropower, the other being that *Holopedium* are also present in Spada

Reservoir, so taking water directly from Spada is not a guaranteed solution to the clogging problem.

The large gelatinous sheath that *Holopedium* exhibits is a very unusual feature among freshwater crustacean zooplankton (Montvilo et al., 1987). It covers the entire carapace, with a slit on the ventral margin which allows the swimming antenna and the filtering appendages contact with the water. The sheath is not attached to the carapace and there is a large gap between the sheath and the dorsal portion of the carapace which can be opened and closed by engaging abdominal bristles (Montvilo et al., 1987). The sheath is close to the refractive index of water, making it effectively invisible in an aqueous environment (Brown, 1970). In a histological study of the sheath, Brown (1970) concluded that the structure is a loose network of cross-linked proteins covalently bound to acidic polysaccharides containing both carboxyl and sulfate groups. Water can flow freely into and out of this network (Brown, 1970). The sheath both increases buoyancy (Hamilton, 1958) and drag (Stenson, 1987). The sheath's primary function appears to be to decrease predation on juvenile *Holopedium*, as the size of the sheath is increased when *Holopedium* are exposed to *Chaoborus kairmones* (Stenson, 1987) and in lakes where predation pressure is primarily on juveniles by ichthyoplankton (Tessier, 1986b). The sheath may also support protozoa or ciliate colonies (Montvilo et al., 1987; Hessen et al., 1990).

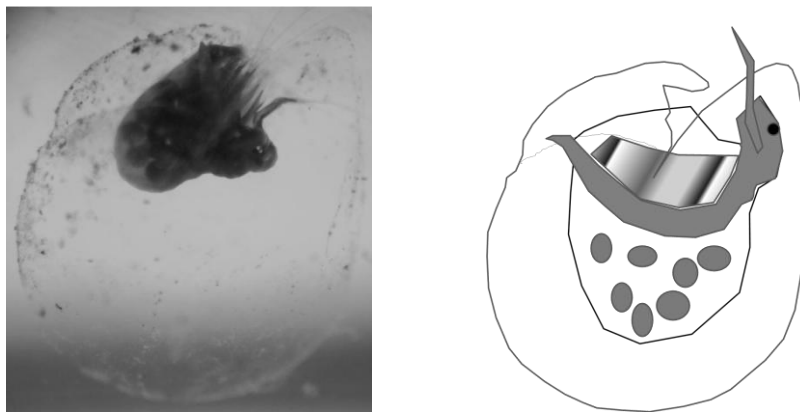


Figure 1: *Holopedium* in sheath and diagram of body orientation of live specimen in sheath

The timing of high *Holopedium* abundance in Lake Chaplain varies from year to year (Figure 2). There is often a spring population peak occurring sometime between March and early June and a fall peak occurring between September and early November. However, in some years

there is no spring population maximum. During 2011 *Holopedium* did not exhibit any population maxima, reaching an observed population of only 0.7 L^{-1} . Variability in timing in *Holopedium* abundance has been found both interannually in lakes as well as between different lakes during a concurrent time period (Tessier, 1986a; Walters et al. 1990; Hessen et al. 1995).

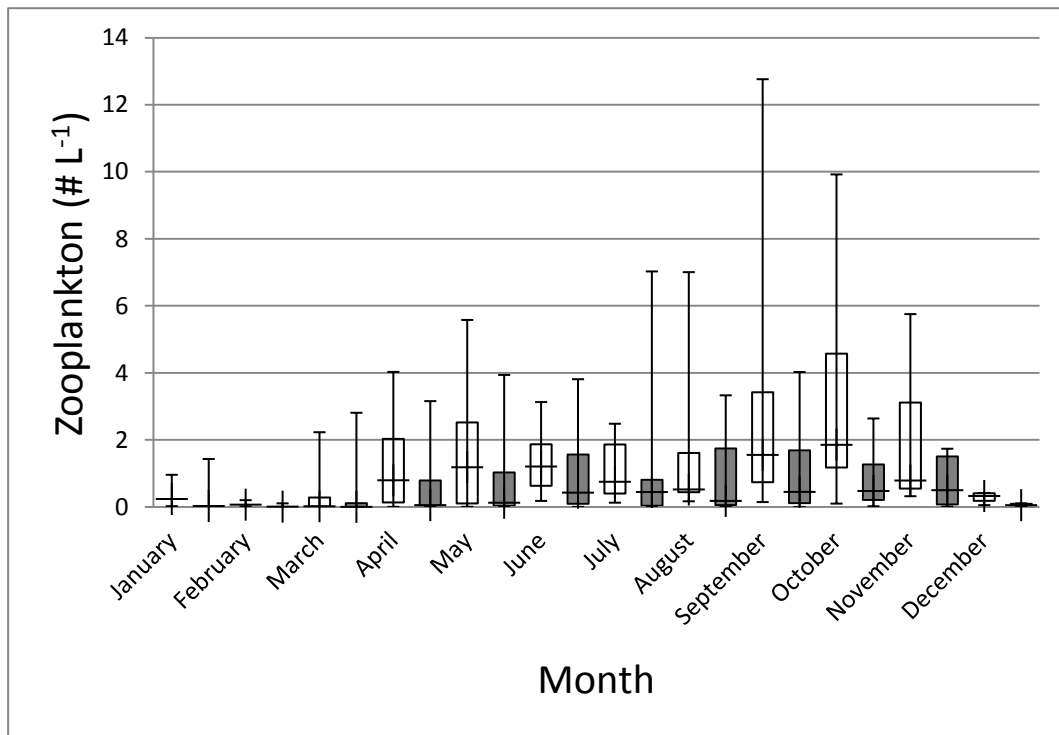


Figure 2: *Holopedium* and *Daphnia* abundances in Lake Chaplain near the Filtration Plant Intakes, 2004-2011 (Grey bars: *Holopedium*, Clear bars: *Daphnia*) Data are from vertical hauls of the full lake depth (12-13m depending on reservoir level). *Holopedium* abundances over 2 L^{-1} are likely to affect plant operation.

Heterogeneity in zooplankton abundance both horizontally and vertically can be due to by many factors (Folt and Burns, 1999). In addition to passive physical transport, zooplankton display several modes of active aggregation and zooplankton are known to change their depth in the water column in a daily pattern (Lampert et al., 1988). This is known as diel vertical migration, with the zooplankton typically being deeper in the lake during the day and close to the surface at night. Diel vertical migration in cladocerans is affected by light and the presence of predator kairomones (VanGool and Ringelberg, 1996; Young and Watt, 1996). Horizontal patchiness in cladocerans can be affected by swimming response to food as well as by predator

abundance (Kleiven et al., 1996). *Holopedium* has been found to most commonly exhibit reverse diel migration in Lake Chaplain, *i.e.* they stay close to the surface during the day and migrate deeper in the water at night. In Lake Chaplain migration is especially pronounced at the beginning of the summer (Spotts and Brett 2008). *Holopedium* restricts its vertical movements in Chaplain primarily to the epi- and metalimnion. In other lakes *Holopedium* has been found to exhibit both regular as well as reverse vertical migration (Rainey et al. 2007; Hoenicke and Goldman, 1987; Tessier, 1986a). However, in some lakes *Holopedium* does not migrate vertically (Gélinas, 2008). Hoenicke and Goldman (1987) suggested that the extent of migration by *Holopedium* might be related to food quality. Under conditions of high food quality about 50% of the *Holopedium* were found at the same depth as the phytoplankton maximum. Hoenicke and Goldman did not find a relationship between predators and the amplitude of migration. *Holopedium* are also known to migrate horizontally in swarms (Tessier, 1983), though this is not be true in all lakes (LeBrasseur and Kennedy, 1972).

Inorganic turbidity in Lake Chaplain varies greatly from year to year, both in timing and intensity (Figure 3). Suspended sediments are known to have various and contradictory effects on zooplankton. Inorganic sediments may interfere with *Holopedium*'s ability to feed by increasing particle rejection rates, clogging filtration appendages and slowing beat rates due to gut fullness (Kirk, 1991). This would result in lower growth rates for *Holopedium*. Alternatively, if bacteria and organics adhere to the inorganic particles, sediment consumption may be beneficial to zooplankton (Jönsson, 2011). The size distribution of the particles can modify their effect on cladocerans. In some cases inorganic particulates have been found to increase zooplankton production, through additional nutrition provided by sorbed organics, more effective digestion of algae due to slower transit through the gut, or better digestion of algae due to mechanical grinding by the sediments (Rellstab, 2007). Due to decreased light penetration, high turbidity may also lower algal production, or may reduce predation on the zooplankton from visual predators such as fish (Kirk, 1990).

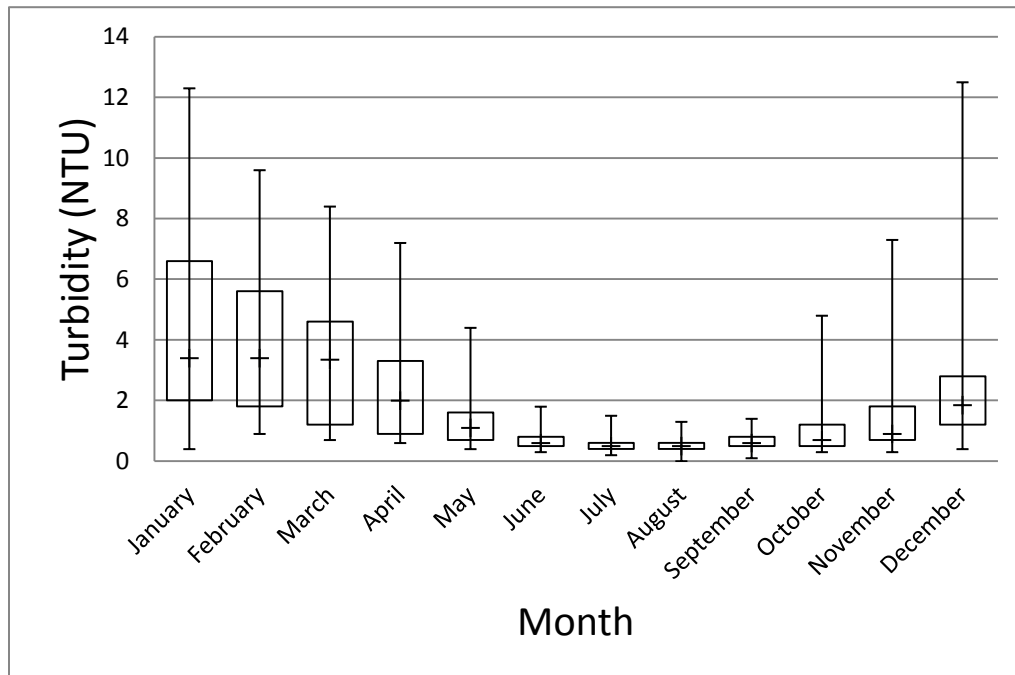


Figure 3: Turbidity at the City of Everett Water Filtration Plant Intakes, 2004-2011. Summary of daily operational measurements

Nutrient levels in a lake affect both the quality and quantity of phytoplankton as a food source for zooplankton. Lake Chaplain is oligotrophic and routinely nitrogen limited during the summer stratified period (Spotts and Brett, 2006) (Figure 4), with chances for co-limitation by phosphorus in July and August (Figure 5). The elemental content of food can limit zooplankton growth (Sterner and Schulz, 1998). *Holopedium* has typical nitrogen and phosphorus demands for a cladoceran, though it has a higher N to P ratio than *Daphnia* (Anderson and Hessen, 1991). In addition to elemental content, food quality is thought to be related to the type and quantity of the fatty acids found in the food (Brett and Müller-Navarra, 1997). Algae such as diatoms or cryptomonads, which contain high levels of the fatty acids most beneficial for cladocerans (Brett and Müller-Navarra, 1997), grow most prolifically when nutrients are not limiting. Cyanobacteria, which are low quality food for *Holopedium* based on fatty acid content, potential toxicity and large colony size, are common when inorganic phosphorus is abundant but inorganic nitrogen is limiting.

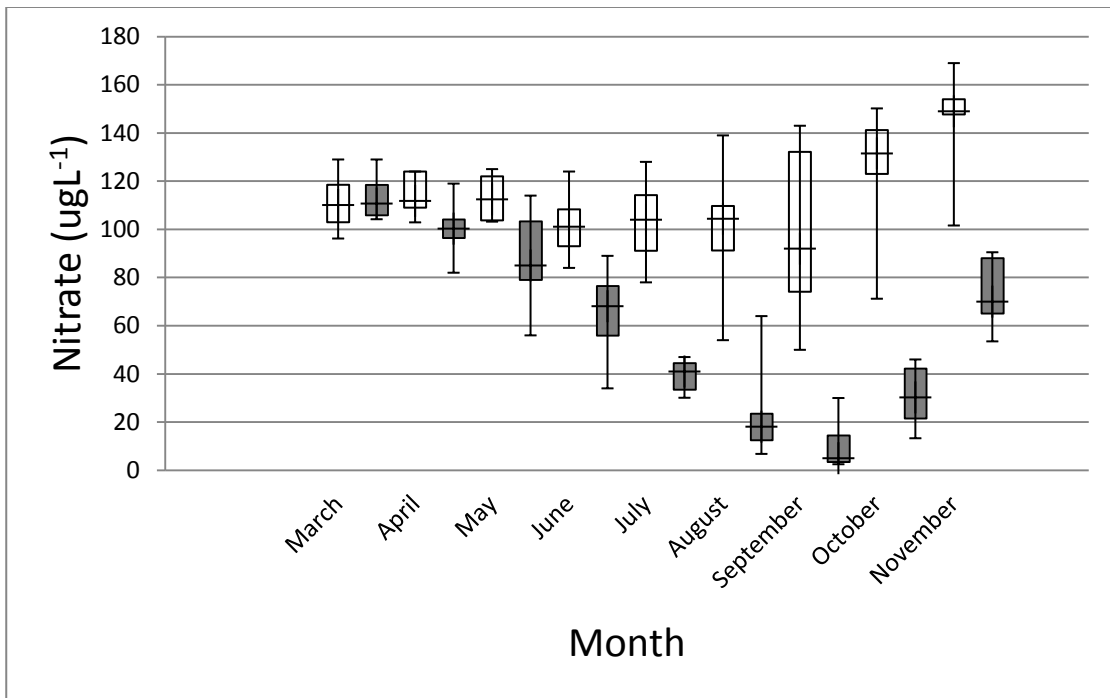


Figure 4: Nitrate concentrations in Lake Chaplain near the center of the lake, 2005-2011 (Grey Bars 1.5m, Clear bars ca. 18m depth) Ammonia is a small fraction of DIN, so nitrate is a reasonable surrogate.

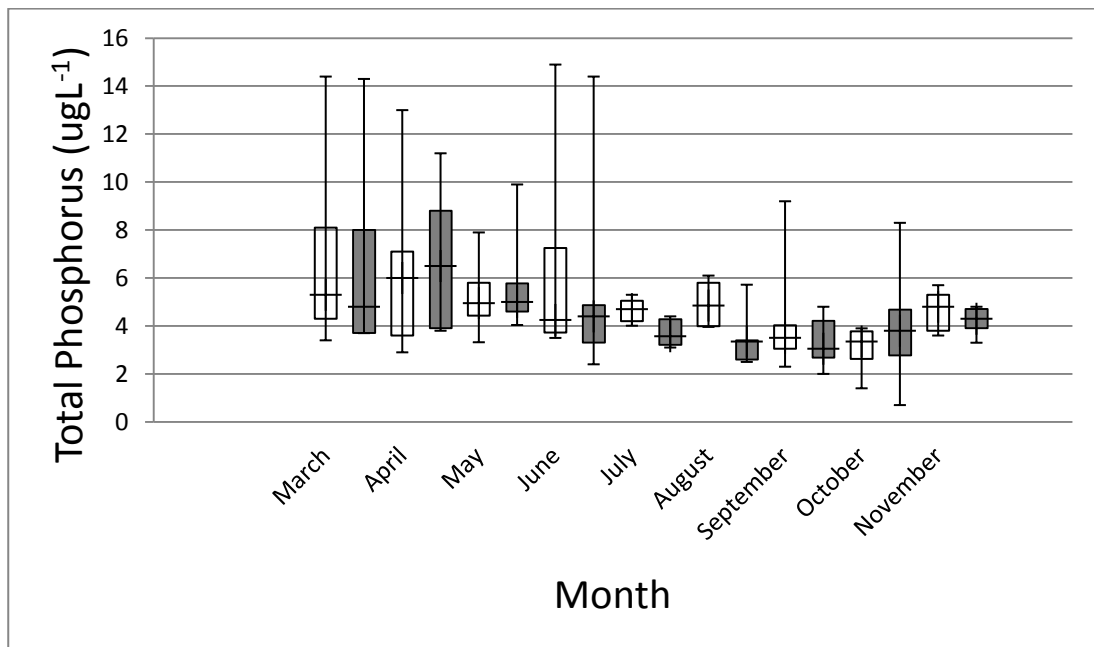


Figure 5: Total phosphorus concentrations in Lake Chaplain, 2005-2011 (Grey bars 1.5m, Clear bars ca.18m). Orthophosphate levels are barely detectable in Lake Chaplain.

Temperature affects the metabolic rates of zooplankton, with higher temperatures permitting higher metabolic rates and therefore higher theoretical growth rates (Bottrell et al. 1976). This pattern can be seen in the finding of Persaud and Williamson (2005); incubation at 8 °C resulted in a smaller adult *Holopedium* population than incubation at 11°C. In the early spring, temperature affects the hatching of resting eggs (Pennak, 1953) with cladoceran resting eggs hatching around 6 °C. *Holopedium gibberum* has an upper threshold for the temperatures it tolerates. Higher temperatures correlate with a decrease in population and fecundity as the water approaches 25°C, above which they disappear from the zooplankton assemblage (Wolfenbarger, 1999). Feeding rates for *Holopedium* have been shown to decline as the temperature increases from 8 to 20 °C in some cases (Walters et al. 1990) though other investigators have found no temperature effect over a similar range (Chow-Frasier and Knoechel, 1985). Temperature can also indirectly influence zooplankton populations as warmer average lake conditions are likely to cause more pronounced stratification and therefore nutrient and food limitation. Temperature conditions in Lake Chaplain near the drinking water treatment plant intakes (12 m depth) tend to be isothermal except during the spring when a thermocline develops and moves deeper in the water column as the season progresses until it reaches the lake bottom, often in July. The center of the lake is deeper (18 m) and exhibits the development of a stable thermocline from June to September or October.

Holopedium and *Daphnia* have substantially overlapping niches (Allan, 1973; Tessier, 1986a; Makarewicz and Likens 1975). Both zooplankters are present in Lake Chaplain for much of the year (Figure 2). Exploitative competition, in which shared food sources become depleted, can occur between *Daphnia* and *Holopedium* during times of high population densities (Allan, 1973). Patterns of food limitation and predation pressures were different for the two species in Tessier's study. *Holopedium* and *Daphnia* have been found to be separated in space and time (Makarewicz and Likens, 1975), thereby minimizing competition for food. Walters et al. (1990) also found no evidence of exploitative competition between *Daphnia* and *Holopedium*. Allan (1973) concluded from feeding studies that *Holopedium* and *Daphnia* compete for algae and detritus with *Daphnia* being the superior competitor, but *Holopedium* likely having a wider feeding niche.

Predation affects *Holopedium* differently than *Daphnia*. Due to the gelatinous sheath, *Holopedium* juveniles do not suffer as much mortality as *Daphnia* from invertebrate predators such as *Chaoborus* (Allan, 1973) and the sheath grows larger when *Chaoborus* are abundant (Stenson, 1987). Tessier (1986a) found that fish also are more likely to prey on *Daphnia* of all size classes than on *Holopedium*. However, Tessier found that ichthyoplankton can significantly decrease the neonates of *Holopedium* and therefore recruitment to the adult stage. In lakes with predation primarily due to ichthyoplankton, *Holopedium* exhibits larger size at birth and maturity and a larger sheath size. In lakes with predation on *Holopedium* by adult fish, both body and sheath sizes are comparatively smaller (Tessier, 1986a). *Chaoborus* is found infrequently and at low densities in Lake Chaplain. Stickleback and trout have been observed in Lake Chaplain, but beyond this, very little is known about the fish community or their effects on zooplankton dynamics and competition between *Holopedium* and *Daphnia*.

Holopedium and *Daphnia* exhibit morphological differences in their filtration apparatuses, suggesting they differ in their ability to utilize different types of phytoplankton. Based on morphology, *Holopedium* has the ability to utilize larger food sizes than *Daphnia*, but is less efficient at utilizing smaller particles, such as bacteria (Geller et al. 1981). This size selection has been confirmed in studies tracking the ingestion of various sized polymer beads by cladocera (Hessen 1985). Hessen et al. (1990) found *Holopedium* to be more efficient than *Daphnia* at utilizing algae but less efficient at utilizing bacteria. The upper end of the effective range of *Holopedium* filtration has been suggested to be a 30-35 μm algal cell, based on changes in the size class distribution of the phytoplankton grazed by *Holopedium* (Cyr, 1999). Based on the studies of Hessen, Geller and Cyr, *Daphnia* have a larger effective range than *Holopedium*, from less than 1 μm up to 75 μm . This could allow *Daphnia* to utilize a broader range of food types.

Experimental observations related to food consumption indicate that *Daphnia* and *Holopedium* consume different resources. Moore (1977) found that the *Holopedium* consumed detritus, sand, and algae, primarily *Cyclotella glomerata* (50-70% total algae in gut contents) and *Chlamydomonas* (5-15% total algae in gut contents). Tessier (1986a) concluded that a large spring bloom of *Chlamydomonas* may have maintained fecundity in *Holopedium*, but not *Daphnia*. Tessier also found that *Ankistrodesmus falcatus* could be used to culture both *Holopedium* and *Daphnia*. Brett et al. (1994) found that *Holopedium* in mesocosms in Castle

Lake decreased the abundance of *Rhodomonas minuta* as well as *Peredinium inconspicuum*. The *Daphnia* in this experiment significantly decreased six other species in addition to the two affected by *Holopedium*, suggesting that the *Daphnia* consumed a wider range of phytoplankton. Brett et al. also found that both *Holopedium* and *Daphnia* have a significant impact on ciliates, with *Daphnia* having the larger effect. It is unclear whether this is due to interference, exploitative competition, or predation. Hoenicke and Goldman (1987) found that seston with abundant diatoms and flagellates could sustain both *Holopedium* and *Daphnia* with no signs of competition, but that *Holopedium* out-competed *Daphnia* later in the summer when chrysophytes were more prevalent. It is likely that competition with *Daphnia* affects *Holopedium* abundance in Lake Chaplain.

Study Objectives

The City of Everett desires to better understand the population dynamics of *Holopedium gibberum* in Lake Chaplain in order to better predict filter clogging episodes. While models can suggest correlations between variables, causative links are harder to establish. Therefore, factors suspected to affect *Holopedium* population dynamics were examined using an experimental approach. These experiments investigated how specific environmental variables influence *Holopedium* bloom onset, development and magnitude. The variables examined relative to *Holopedium* population dynamics were inorganic turbidity, temperature, food sources, and resource competition with *Daphnia*. The effects of inorganic turbidity, temperature and food availability were investigated using *in-situ* single variable experiments. Fatty acid analyses were used to determine if food resources were being utilized differentially by *Daphnia* and *Holopedium* under natural lake conditions.

Based on literature findings and anecdotal observations, it was hypothesized that high levels of inorganic turbidity would decrease *Holopedium* abundance. *Holopedium* were expected to thrive under conditions in which nutrients and temperature do not limit the quantity or quality of algae as a food source. Temperature was expected to limit *Holopedium* populations due to constraints on metabolism, even if food was abundant. *Holopedium* and *Daphnia* were predicted to utilize overlapping food sources, leading to competitive exploitation under conditions when food is limited and these species overlap spatially and/or temporally.

Methods

In-Situ Experiments

Cubitainers were filled with lake water collected with a Van Dorn sampler below the lake surface and filtered through a 233 μm net to remove large zooplankton. All cubitainers were randomly filled in two steps to minimize differences in water quality between cubitainers. Zooplankton were obtained from the lake using a 233 μm net. *Holopedium* were selected using inoculation loops and transferred into zooplankton-free lake water. The liveliest *Holopedium* were transferred to the cubitainers. The number of individuals used in each treatment varied from 1-2.5 L^{-1} , depending on the availability of *Holopedium* in the lake at the time of the experiment (Table 1). The cubitainers were suspended at the desired depth (3-13 m, Table 1) at or near the middle of Lake Chaplain, and incubated for 7-10 days.

Turbidity was investigated using blue clay obtained from the Spada Reservoir watershed. Clay was mixed with water from the filtration plant intakes, *ca.* 12 m. The mixture was then allowed to settle for ninety minutes prior to the supernatant being used in the set up for Experiment 3 and was subsequently left undisturbed and the supernatant used again in the set up for Experiment 4 (Table 1).

Food and temperature interactions were investigated by spiking cubitainers with 50 $\mu\text{g L}^{-1}$ of P (PO_4) and 300 $\mu\text{g L}^{-1}$ of N (NO_3) and suspending them in the lake to incubate for a week prior to the experiment, thus allowing an algae bloom to develop in the cubitainer. 900 mL of the contents of this cubitainer were added along with nutrients at the same concentrations as above for the nutrient enriched treatments. To vary temperature, the cubitainers were suspended just above and just below the thermocline (*ca.* 10 and 13m), where the average temperatures for the duration of the experiment were 16 and 9 $^{\circ}\text{C}$ respectively (Table 1).

After 7-10 days the cubitainers were removed from the lake, brought back to the lab and their contents filtered through a 125 μm net to capture the zooplankton, which were subsequently preserved with Lugol's solution. The filtrate was analyzed for several parameters depending on the experimental set-up. Chlorophyll was filtered immediately on Whatman G/F filters, frozen, and analyzed at the University of Washington (UW) limnology lab (Turner Designs 10-AU Fluorometer). Turbidity was measured on a HACH 2100N turbidimeter. Phytoplankton samples were collected, preserved with Lugol's solution, and analyzed at Water Environmental Services or at Nostoca Algae Laboratories. The zooplankton samples were analyzed for number of

Holopedium as well as any other zooplankton species present. The number of gravid *Holopedium* and number of eggs per gravid individual were also noted. The samples were then photographed and the *Holopedium* were sized using the computer program ImageJ (Abramoff, 2004). The area measurements obtained from ImageJ were converted to biomass using the equation obtained by regressing known dry weights of sized classed *Holopedium* with the area results from the image analysis of the same organisms.

$$\text{Mass } (\mu\text{g}) = 37.41 * \text{SA}^{1.3209} \quad \text{where SA} = \text{area of image in mm}^2 \quad (\text{Eq. 1})$$

In-Situ Statistical Analysis

The results from each cubitainer experiment were analyzed using MANOVA with IBM-SPSS Statistics version 20. Parameters which demonstrated significant differences were further investigated using Student t-tests between the single variable treatments. Significance was set at $\alpha < 0.05$ for all statistical tests. Almost significant refers to values between $\alpha = 0.05$ and $\alpha < 0.10$.

Table 1: *In-situ* Experimental Set-up

Experiment #	Date Deployed	Date Retrieved	Incubation Depth (m)	Temperature (°C)	Added Food	Added Turbidity (NTU)	Initial # of <i>Holopedium</i> *L ⁻¹	Number of Replicates
1	08/09/11	08/18/11	3	17	No	No	2.5	5
1	08/09/11	08/18/11	3	17	Yes	No	2.5	5
2	08/24/11	09/01/11	10	16	No	No	2.0	5
2	08/24/11	09/01/11	10	16	Yes	No	2.0	5
2	08/24/11	09/01/11	13	9	No	No	2.0	5
2	08/24/11	09/01/11	13	9	Yes	No	2.0	5
3	09/07/11	09/16/11	10	17	No	15	2.0	5
3	09/07/11	09/16/11	10	17	No	8	2.0	5
3	09/07/11	09/16/11	10	17	No	No	2.0	5
4	09/20/11	09/27/11	10	16	Yes	No	1.1	5
4	09/20/11	09/27/11	10	16	No	No	1.1	5
4	09/20/11	09/27/11	10	16	Yes	10	1.1	5
4	09/20/11	09/27/11	10	16	No	10	1.1	5

Fatty Acid Sample Collection

Samples were collected for seston fatty acid analysis concurrently with phytoplankton in

Lake Chaplain, near the center of the lake. The samples were composited from water taken with a Van Dorn sampler 0.5 m below the surface and every meter and a half below that, to one meter above the lake bottom which was between 19-20 m during the study. These samples were then returned to the lab, filtered through a 233 μm zooplankton net to remove large bodied zooplankton, and then filtered through pre-combusted Whatman G/F filters. The filters were then kept frozen at $-20\text{ }^{\circ}\text{C}$ until analysis. Samples of *Daphnia* and *Holopedium* were obtained with a zooplankton net, vertical tow, near the center of the lake, except in one instance of low *Holopedium* abundance when a horizontal tow was used. These samples were brought back to the lab live where 3-40 individual *Daphnia* and *Holopedium* were picked using inoculation loops. The samples were kept frozen at $-20\text{ }^{\circ}\text{C}$ until analysis.

Fatty Acid Analysis

Samples were freeze-dried for 24 h (Labconco Freezone 4.5). Zooplankton samples were then weighed (Cahn model C33 Microbalance). Fatty acids were extracted using a chloroform:methanol:water mixture (Parrish, 1999), followed by methylation with a 1% sulfuric acid-methanol reagent in the presence of toluene, addition of potassium bicarbonate and subsequent extraction using hexane/diethyl ether with 1% BHT. The samples were then evaporated to dryness and re-suspended in Gas Chromatography (GC) grade hexane.

Analysis of fatty acids was carried out in the UW limnology lab on a HP 6890 GC equipped with a J&W Scientific DB-23 column (30m x 0.25mm, 0.15 μm film thickness), a flame ionization detector and helium as the carrier gas. One μL of sample was injected. The following 85 minute temperature program was used: $50\text{ }^{\circ}\text{C}$ held for five minutes, ramp at $10\text{ }^{\circ}\text{C}$ per minute to $100\text{ }^{\circ}\text{C}$, ramp at $2\text{ }^{\circ}\text{C}$ per minute to $140\text{ }^{\circ}\text{C}$, ramp at $1\text{ }^{\circ}\text{C}$ per minute to $180\text{ }^{\circ}\text{C}$, hold for five minutes, ramp at $2\text{ }^{\circ}\text{C}$ per minute to $200\text{ }^{\circ}\text{C}$ and finally ramp at $10\text{ }^{\circ}\text{C}$ per minute to $240\text{ }^{\circ}\text{C}$. Peaks were identified based on a 37 Supelco standard from Sigma Aldrich, a Nucheck 40 Standard and from past data gathered on the instrument and verified on Gas Chromatography-Mass Spectroscopy (GC-MS) by Aaron Galloway (unpublished data). Response functions were semi-quantified based on serial dilution of a Nucheck 40 Standard.

Fatty Acid Statistical Analysis

The fatty acid results were arcsine square root transformed and then analyzed by

principle component analysis (PCA) using IBM-SPSS Statistics version 20. The total fraction of individual fatty acid constituents were summed according to chain length, as well as number and position of double bonds, yielding 15 groups as follows, all saturated fatty acids (SAFA); C₁₄, C₁₆, C₁₈ and C₂₄ monounsaturated fatty acids (MUFAs); C₁₆, C₁₈, C₂₀ and C₂₂, ω₃ and ω₆ polyunsaturated fatty acids (PUFAs); and other C₁₆ and C₁₈ FAs. PCA was run on these values for all the data as well as a subset containing only the values for *Daphnia* and *Holopedium*. Data that either did not score above 0.5 for communality or that showed complex structure in the varimax rotated component matrix was systematically excluded from analysis. Correlations between FA composition of *Holopedium*, *Daphnia*, and seston were investigated using the Student t-test and regression analysis of the arcsine transformed fatty acid (FA) composition data. Semi-quantified data was compared with phytoplankton biovolume data using Spearman's Rho.

Concurrent Lake Monitoring

As part of the routine monitoring program on Lake Chaplain, sampling was conducted every two weeks during the growing season in 2011 (March-November) for multiple parameters at two or three sites, including a site close to the treatment plant intakes, and a site near the center of the lake. The field parameters measured included an *in-situ* vertical profile of temperature, conductivity, chlorophyll, turbidity, dissolved oxygen, pH using a YSI 6600 data sonde at three meter intervals. Laboratory analyses included chlorophyll, total and dissolved nutrients at a single depth from the epilimnion and hypolimnion (usually *ca.* 2 m below the surface and *ca.* 2m above the bottom) analyzed by the UW Oceanographic Laboratory, a vertical profile of phytoplankton analyzed by Water Environmental Services, and a vertical profile of zooplankton analyzed by City of Everett staff.

In-Situ Experiment Results

Experiment 1

Experiment 1 was designed to test both the method of cubitainer set-up and the effects of nutrient enrichment. Statistics were run on the portion of the experiment in which the cubitainers were set up as in subsequent experiments. The average number of eggs and percent gravid individuals was significantly higher in the nutrient enrichment treatment. Chlorophyll

concentration was also significantly higher in the nutrient enrichment treatment.

Experiment 2

Experiment 2 tested the effects of nutrient enrichment and incubation temperature. The number of *Holopedium*, the percent gravid individuals and the average mass per individual were all significantly different between treatments. Differences in total mass were almost significant. Temperature had a large effect on the response variables. In contrast, nutrient enrichment only affected the chlorophyll concentrations. Nutrients and temperature had significant interaction effects on average *Holopedium* mass and almost significant effects on total mass. Temperature alone had no effect on chlorophyll. The interaction between nutrients and temperature resulted in the warm, food enriched conditions yielding the strongest treatment responses in post-hoc comparisons. Colder temperatures limited *Holopedium* growth more than low food levels did. Nutrient enrichment affected total biomass production more than temperature did.

Experiments 3 and 4

Experiments 3 and 4 were intended to test the effects of turbidity on *Holopedium*. None of the response variables were significantly different between the treatments, even when nutrient addition was employed to enrich food conditions.

In-Situ Experiment Discussion

Nutrient Addition

Under warm conditions, such as Experiment 1 and the warm incubation in Experiment 2, nutrient enrichment had a different effect than under cold conditions. In both warm experiments nutrient addition increased the number of eggs and the percent gravid individuals. In Experiment 2, the warm incubation also increased both average and total mass but did not increase the number of individuals, indicating that the increase in mass was primarily due to more eggs. Under cold conditions, the only significant effect was an increase in chlorophyll. Food enrichment had no effect on *Holopedium* at colder incubation temperatures (8 °C), presumably due to temperature limits on metabolism. The increase in fecundity with food indicates that the zooplankters' metabolic rate was sufficiently high at warmer temperatures (16 °C) to allow utilization of the available food resources. These results indicate that ideal food under cold

temperatures may result in less growth and recruitment than non-ideal food under warm conditions, similar to the findings of Park et al. (2004).

Incubation Temperature

There were twice as many *Holopedium* on average in the experiments exposed to warm water than those incubated at cold temperatures. The individuals in the warm experiments were likely juveniles as they were on average less than half the size and four times less likely to be carrying eggs than the individuals in the cold treatments. The total biomass between the containers was not significantly different. These results indicate that recruitment was accelerated in the warmer conditions, yielding a younger, smaller, and more numerous population. Persaud and Williamson (2005) also found more individuals under warmer conditions in enclosure experiments with *Holopedium*, although they noted an increase in adults and not juveniles.

Turbidity

Unfortunately, due to the late summer timing of the experiments related to turbidity and the use of water from two to three meters to fill the cubitainers, these experiments likely produced results that reflected the experimental set-up rather than the intended experimental variable. Nutrient restriction was more severe later in the season in the lake water used to fill the cubitainers (TP at two meters decreased from 14 to 2 $\mu\text{g L}^{-1}$, Nitrate from 65 to 4 $\mu\text{g L}^{-1}$). The final chlorophyll values and phytoplankton biovolumes in the cubitainers were an order of magnitude smaller in Experiment 4 (September) than in Experiment 2 (August). The quality of the food in the lake water likely decreased over the course of the summer. Pyrrophyta made up a smaller fraction of the biovolume in the later experiments. The greatest cyanobacteria biovolume of any experiment was present in the nutrient-turbidity cross experiment from late September. Even the treatments with nutrient enrichment fared worse with regard to phytoplankton growth later in the season. Anecdotal evidence from Lake Chaplain indicates that high turbidity favors *Daphnia* over *Holopedium*, but the mechanism for this is not clear. It would be interesting to repeat these experiments again during early summer lake conditions, when the food quantity and quality in the lake water is higher.

Changes in Phytoplankton

Though there was no experimental control for the phytoplankton, it is useful to look at the shifts in biovolume and taxa from the beginning to the end of an experiment. Over the duration of Experiment 1, the phytoplankton biovolume doubled in the cubitainer filled with lake water and more than quadrupled in the cubitainer with nutrients. It is counterintuitive for the total biovolume of phytoplankton to increase under grazing pressure. However, the fraction edible by *Holopedium*, assuming an effective range of 5-35 μm , decreased by half in the lake water treatment. In the nutrient enriched experiment, this fraction increased twofold over the course of the experiment, but the fraction of the total biovolume that was edible by *Holopedium* decreased in both cases.

In the lake water treatment, while the biovolume of Cyanophyta, and Chlorophyta increased over the course of the experiment, Cryptophyta decreased. Under food enriched conditions the chrysophytes increased threefold while the cyanophytes, cryptophytes, and pyrrhophytes decreased. Changes in the fraction of the phytoplankton available to *Holopedium* followed slightly different patterns. The biovolume of the edible size fraction of Chlorophyta and Chrysophyta increased the most in both treatments. Edible cryptophytes and pyrrhophytes decreased in the lake water and barely increased in the nutrient treatment. The edible sized Pyrrhophyta that did increase consisted of dinoflagellates with mucous envelopes; because of this, they were likely not very edible. *Rhodomonas sp.* disappeared completely in both treatments (originally around 7% of the biovolume) and *Peridinium sp.* which was originally around 20% of the biovolume and dropped to less than 3%. Despite the lack of a control treatment designed to assess phytoplankton response to zooplankton grazing, the results of this analysis are similar to the findings of Brett et al. (1994) in which *Holopedium* grazing significantly decreased only *Peridinium sp.* and *Rhodomonas minuta* in controlled microcosm experiments.

Implications for Holopedium Dynamics in Lake Chaplain

The results of the cubitainers indicated that when *Holopedium* are exposed to average temperatures *ca.* 16 °C they are expected to be able to double their numbers twice as fast as when they are exposed to temperatures *ca.* 8 °C. Given the same amount of food, population growth rates would be twice as fast in Lake Chaplain in the end of August than in mid May. The temperature at which food quantity starts to have an effect was not determined by these

experiments. It would be useful to determine at what temperature food will increase exponential growth by increasing the average population fecundity as was seen in the warm cubitainers. This could be accomplished by starting cubitainers earlier in the season when the water is still cooler in the epilimnion or by short term experiments in the lab at different temperatures. It may be that at cold temperatures, metabolic rates are too low to take full advantage of the available food.

The timing and depth of the spring bloom, total summer heating of the water column, and the timing of fall turnover will influence the temperature experienced by *Holopedium* during periods of abundant food. A cold summer could result in a fall turnover phytoplankton bloom having little effect on the *Holopedium* population as the water would be relatively cold, whereas early turnover after a warm summer could allow for larger population increases. A spring bloom in early April might be expected to produce lower numbers of *Holopedium* than a spring bloom in early June due to differences in the speed of population growth and ability to utilize the available resources to support recruitment.

Fatty Acid Results

Averages and Standard Deviations

In both *Holopedium* and *Daphnia* ω 3 PUFAs (see Table 2 for abbreviations) were the most prevalent FAs with EPA being the most common. SAFAs were the most prevalent FA in the seston with MUFAs close behind. In *Daphnia* SAFA and MUFA levels were about equivalent, whereas *Holopedium* had more SAFAs than MUFAs. *Daphnia*, seston and *Holopedium* had oleic acid as their most prevalent MUFA. In all three groups 16:0 was the most common SAFA, followed by 14:0. The least prevalent FAs in all three groups were the ω 6 PUFAs with LIN being the most common for *Daphnia* and seston and ARA for *Holopedium*. *Daphnia*'s total fat content was much more variable than that of *Holopedium* and the seston; however, the seston showed the greatest variability in relative FA composition.

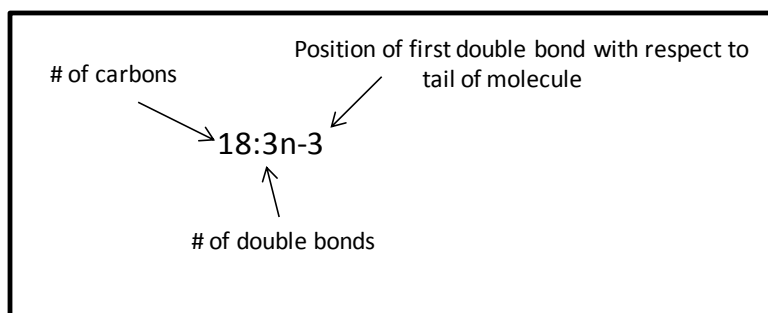


Figure 6: Fatty acid naming convention

Table 2: Fatty acid nomenclature and structure as used in this study

Abbreviation	Full Name	Structure	Biological Relevance for Cladocerans
FA	Fatty Acid		
MUFA	Monounsaturated Fatty Acid	single double bond	
$\omega 3$ PUFA	Polyunsaturated Fatty Acid	multiple n-3 double bonds	
$\omega 6$ PUFA	Polyunsaturated Fatty Acid	multiple n-6 double bonds	
Oleic	Oleic acid	18:1n-9	
LIN	Linoleic acid	18:2n-6	precursor to ARA
ALA	α -Linolenic acid	18:3n-3	precursor to EPA
SDA	Stearidonic acid	18:4n-3	precursor to EPA
ARA	Arachidonic acid	20:4n-6	cell membranes composition, hormone precursor
EPA	Eicosapentaenoic acid	20:5n-3	cell membrane composition, reproduction, hormone precursor
DHA	Docosahexaenoic acid	22:6n-3	precursor to EPA

Paired t-tests

Paired t-tests between seston, *Daphnia*, and *Holopedium* FA composition revealed significant differences with respect to the SAFA, MUFA, $\omega 3$ PUFA, and $\omega 6$ PUFA FA groups. Seston had the most SAFA and *Daphnia* the least, and *Holopedium* had SAFA levels indistinguishable from seston. *Holopedium* had the least MUFA and seston the most, with

Daphnia being more similar to the seston. Seston had the least and *Holopedium* the most ω 3 and ω 6 PUFAs. *Daphnia* exhibited PUFA levels more like those of *Holopedium* than those of the seston. However, the most prevalent FAs within the groups mentioned above were not always distinguishable between *Holopedium*, *Daphnia*, and seston. All three were distinguishable with regards to 14:0 and but not 16:0. *Holopedium* had the most 14:0, just slightly higher than seston. *Daphnia* had just over half of the 14:0 of seston. Seston had the most 16:0 and *Holopedium* the least, with *Daphnia* being indistinguishable from the seston. For the MUFAs, *Daphnia* had more oleic acid than the seston, but *Holopedium* had significantly less. Among the ω 3 PUFAs, EPA was distinguishable between all groups with seston having the least and *Holopedium* the most and *Daphnia* being more similar to *Holopedium*. The amount of the ω 6 PUFA LIN was indistinguishable between *Holopedium* and seston, but significantly higher in *Daphnia*. ARA was not present in the seston and *Holopedium* had significantly more of this ω 6 FA than did *Daphnia*.

Principle Component Analysis

Principle component analysis (PCA) among the FA composition of all three groups showed that 98% of the variance in the fatty acid composition can be explained by a single component made up of ARA and EPA. This reflects the lack of ARA in the seston and the large difference between the proportions of EPA in the cladocerans compared to the seston. A PCA among *Daphnia* and *Holopedium* alone showed that 81% of the variance can be explained using a single component comprised of the C₁₈ MUFA s and the C₁₈ ω 6 FAs. *Daphnia* contained more of these two FA groups than *Holopedium*.

Correlations between Cladocerans and Seston Fatty Acids

Daphnia's FA profile correlated significantly with that of the concurrent seston profile for eleven FAs whereas *Holopedium* correlated significantly with five. Compared to the seston profile two weeks prior, *Daphnia* correlated with eight individual FAs and *Holopedium* with six, the shift for *Holopedium* occurring entirely from correlations in the C₁₆ to the C₁₈ series (18:0, oleic, LIN, ALA and SDA). Concurrent profiles of Seston and *Daphnia* correlated most strongly with 24:1n-7 and LIN ($r^2 \approx 0.80$) as well as EPA. Oleic acid and 16:0 were also correlated between the seston and *Daphnia* with $r^2 \approx 0.60$. Concurrent profiles for seston and *Holopedium*

had weaker correlations with the seston than those between seston and *Daphnia*. However, several correlations were significant, the strongest correlation being with 16:0. Between the seston FA profiles and the *Holopedium* profiles sampled two weeks later, 18:0, oleic, and LIN had the best correlation ($r^2 \approx 0.50$). *Holopedium* and *Daphnia* correlated most with the minor FA, 16:1n-7t. However, they also had significant correlations for some major FAs including oleic, LIN and ALA ($r^2 \approx 0.60$) both in concurrent profiles as well as in the comparison of *Holopedium* to *Daphnia* sampled two weeks prior. The ratio of $\omega 3$ PUFA / $\omega 6$ PUFA correlated between all three groups.

Fatty Acid Temporal Trends

Some of the FAs mentioned above exhibited seasonal trends in percent composition in the seston that were mimicked in the cladocerans (Figure 7). The MUFA oleic acid has a strong midseason maximum for both the seston and *Daphnia*. The $\omega 6$ PUFAs had a July maximum in the seston, with both *Holopedium* and *Daphnia* having a maximum two weeks later. LIN showed a maximum in July for both seston and *Daphnia*. *Holopedium* had a much subtler August maximum with a late season decrease similar to *Daphnia* and the seston. The $\omega 3 / \omega 6$ ratio in seston, *Holopedium*, and *Daphnia* exhibited a minimum in late July, with higher values early and late season.

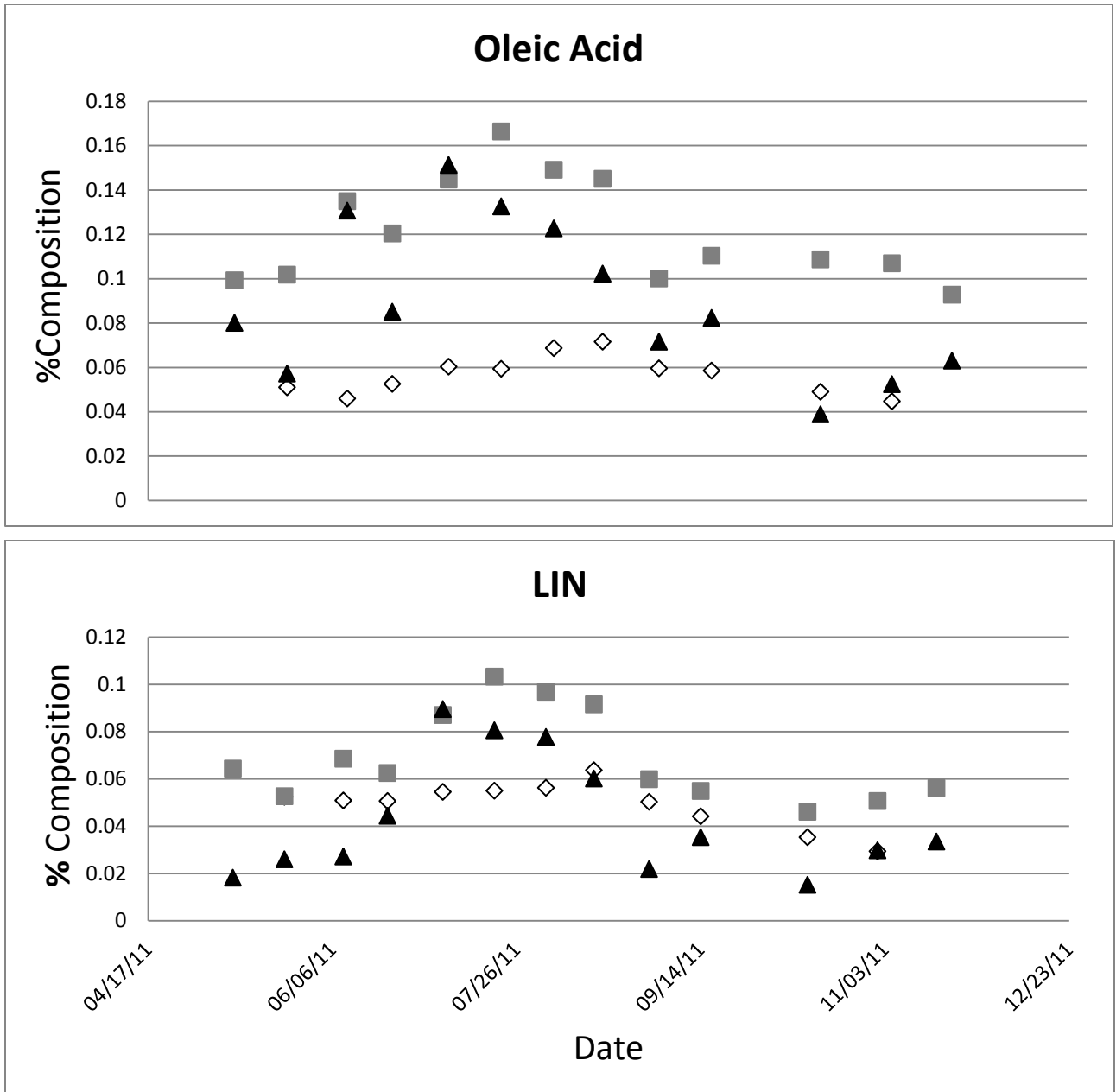


Figure 7: Temporal trends in oleic acid and LIN in the seston and the cladocerans (Seston: black triangles, *Daphnia*: grey squares, *Holopedium*: white diamonds)

Temperature and Fatty Acid Profile Correlations

Significant inverse correlations between water temperature and the seston FA profiles were found for 14:1n-5. *Daphnia* showed an inverse relationship between water temperature and SDA with an r^2 of 0.52. *Holopedium* demonstrated several strong relationships between water temperature and their FA profiles. Oleic acid, 18:1n-7 and ARA all similarly increased with temperature with r^2 s of 0.54, 0.62, and 0.87, respectively. ALA and SDA both decreased with temperature with r^2 s of 0.71 and 0.76, respectively.

Phytoplankton Taxa and Fatty Acid Profiles

Seston SDA demonstrated a significant Spearman's Rho with the Cryptophyta biovolume. Given that SDA is known to be linked to Cryptophyta in the literature as well (Table 3), it is likely a good biomarker for Cryptophyta in Lake Chaplain. Pyrrophyta, the other taxon known to contain large quantities of SDA, correlated significantly with LIN in the seston instead. However, this correlation may be spurious as it has not been previously observed. The sum of $\omega 3$ and $\omega 6$ PUFAs in the seston did not correlate with the total phytoplankton biomass.

Fatty Acid Discussion

Overview

Holopedium and *Daphnia* respond differently to the FAs in the seston in Lake Chaplain, indicating underlying differences in resource utilization and physiology related to resource specialization. *Daphnia* responds to a broad range of FAs on a shorter timescale than *Holopedium* indicating a wider niche. *Holopedium* seems to feed on a smaller portion of the seston and have greater capacity for bioconverting FAs than *Daphnia*, which would make it well suited to survive in oligotrophic lakes where the resource input from allochthonous sources is relatively high and the algae are often dominated by chrysophytes.

General Conclusions

Correlations between seston and cladoceran FAs were primarily among the FAs with known physiological significance for cladocerans, *e.g.* for those critical to cellular membrane composition or for reproduction. However, *Daphnia* also correlated strongly with a few non-essential seston FAs. *Daphnia* and *Holopedium* were significantly different with respect to

SAFA, MUFA, ω 6 PUFA, and ω 3 PUFA content. *Holopedium* demonstrated a longer FA turnover time than *Daphnia*. *Holopedium* also demonstrated more temperature dependency in FA content. Total and individual FA values for both *Holopedium* and *Daphnia* were within previously published ranges for these taxa. This is the first study in which *Holopedium* FA content demonstrated a correlation with seston FA content, and one of the few in which *Daphnia* FA content in an oligotrophic system was shown to reflect seston FA content.

Fatty Acid Correlations with Phytoplankton

Lake Chaplain has an abundance of chrysophytes. Diatoms are also common, especially during early season stratification. Chlorophyta, which is absent early season, appears in the warmer water months. Pyrrhophyta also is more abundant during the mid summer. The phytoplankton present during this study (2011) can be seen in Figure 8.

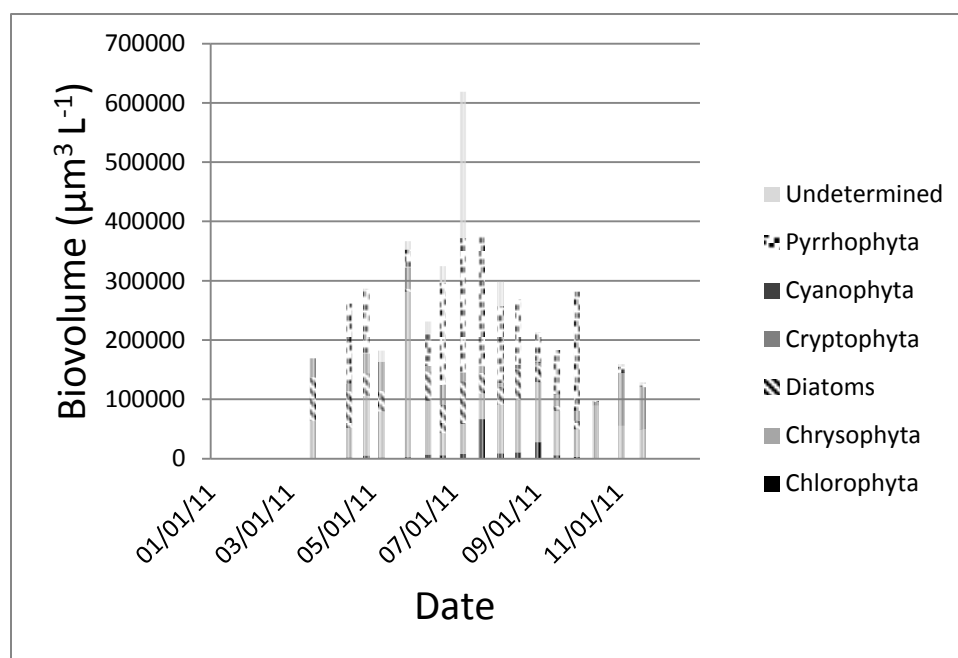


Figure 8: Phytoplankton taxa in Lake Chaplain near the center of the lake, 2011


Diatoms were positively related to 14:0 in *Holopedium* at a two week offset. In terms of contribution to the total FA content, 14:0 and EPA are more prominent in *Holopedium*, likely indicating a reliance on diatoms or chrysophytes greater than that of *Daphnia* (Table 3). The diatom marker 16:1n-7c was of equivalent importance in the two cladocerans. Oleic acid, as well

as LIN, was more important in *Daphnia* than in *Holopedium*, especially mid-summer (Figure 7). SDA was more abundant in *Holopedium*, but also found consistently in *Daphnia*, thus it is likely both cladocerans utilize cryptophytes. That the seston ω 3 and ω 6 PUFA content did not correlate well with the total phytoplankton biomass suggests that there may be other important sources of PUFAs in the lake, such as terrestrial plant material.

Table 3: Characteristic fatty acids of possible seston components in Lake Chaplain

Compiled from: Ahlgren (1992), Bec et al. (2010), Brett et al. (2006 and 2009), Chu et al. (2009), Cranwell et al. (1988), Desvillettes and Bec (2009), Kagami et al. (2007), Leblond and Chapman (2000), Mascalaux et al. (2011), Parrish et al. (2000), Piotrowska-Seget and Mroziak (2003), Taiple et al. (2009), Vera et al. (2001)

Fatty Acid	Chlorophyta	Chrysophyta	Diatoms	Cyanophyta	Cryptophyta	Pyrrhophyta	Heterotrophic Protists / Ciliates	Fungi	Gram Positive Bacteria	Gram Negative Bacteria	Methane Oxidizing Bacteria	Terrestrial Material
c14:0		■		■	■		■				■	
i15:0				■					■			
i17:0, a15:0, a17:0									■			
c16:0	■	■	■	■	■	■	■	■	■	■	■	■
16:1n8c											■	
16:1n7t										■	■	
16:1n7c		■	■	■	■		■	■		■	■	
16:1n-5c								■			■	
16:3n-3	■							■				
16:4n-3	■											
i18:0	■								■			
c18:0			■	■	■		■	■				
18:1n-9 (oleic)	■	■		■	■		■	■				■
18:1n-7												
18:2n-6 (LIN)	■	■		■	■		■	■				■
18:3n-3 (ALA)	■				■			■				■
18:4n-3 (SDA)		■			■		■					
18:5n-3						■						
20:4n-6 (ARA)			■	■	■		■					
20:5n-3 (EPA)		■	■	■	■		■					
22:6n-3 (DHA)		■	■		■		■					
>24:0								■				■


 Dominant or Characteristic Component
 Prevalent
 Trace or Uncommon

Differences in Food Utilization

Holopedium may be able to utilize chrysophytes and the chlorophytes as a source of food more effectively than *Daphnia* (Tessier, 1986a; Hoenicke and Goldman, 1987). ARA and EPA content are very low to non-existent in both chlorophytes and chrysophytes, but the precursors for ARA (*i.e.* LIN) and EPA (*i.e.* ALA) are present in both taxa. *Holopedium* accumulates more ARA and EPA than *Daphnia*, which could function to sustain them during periods of exposure to food lacking these essential FAs. It could also reflect a more active bioconversion of ARA and EPA from LIN and ALA; giving *Holopedium* a competitive advantage during periods when the ARA and EPA content in the seston is low. Hoenicke and Goldman (1987) found that the competitive advantage for *Holopedium* was not apparent when diatoms and flagellates, which are rich in ARA and EPA, were abundant, further supporting the idea that *Holopedium* may have an advantage during times when bioconversion is necessary to supply adequate quantities of these essential FAs. By extension, *Holopedium* might be able to utilize terrestrially derived material (high in LIN and ALA, low in ARA and EPA) more effectively than *Daphnia*, especially after it has been trophically upgraded by fungi or bacteria (Kagami et al., 2007; Masclaux et al., 2011). The existence of a food source adequate in essential FAs for *Holopedium*, but not for *Daphnia* would be in accordance with the observation that *Holopedium* has a wider niche than *Daphnia* (Allen, 1973).

Matthews and Mazumder (2006) found that *Holopedium* became more dependent on allochthonous carbon than *Daphnia* following thermal stratification. They conclude that this reflects habitat specialization, with *Holopedium* found above the hypolimnion and *Daphnia* below it, a pattern also noted by Makarewicz and Likens (1975) and LeBrasseur and Kennedy (1972). Spotts and Brett (2008) found that *Holopedium* stayed above the hypolimnion in Lake Chaplain but *Daphnia* were found both above and below the thermocline. The more numerous correlations between *Daphnia* and seston FAs than between *Holopedium* and seston could reflect a greater access to the full range of food sources in the seston that would come with larger migration amplitude. The seasonal discrepancy in the uptake of LIN and oleic acid between the two cladocerans corresponds to the stratified summer period when the chlorophyll maximum was in the hypolimnion rather than the epilimnion. A deep chlorophyll maximum is often attributed to nutrient availability, but could also be the result of grazing in the epilimnion by mixotrophic phytoplankton (Tittel et al, 2003), such as chrysophytes, pyrrhophytes or cryptophytes.

Turnover of Fatty Acids

The total FAs measured in the cladocerans are a combination of the FAs in the body tissue (cell membrane), lipid stores, and eggs. The degree of correspondence with concurrent seston FA content will depend on the rate at which these three FA pools turn over. Taipale et al. (2011) found that *Daphnia* cell membranes are comprised of stearic and oleic acid, LIN, ARA and EPA. The turnover times of membrane and total FAs in Taipale's study were similar. It took two days to replace 50% of the FA content and six to reach a new equilibrium in diet switching experiments carried out at 19 °C. It is quite likely that in natural systems this time may be longer due to lower metabolic rates in zooplankton corresponding to the lower temperatures found in most lakes. The turnover period will also vary from species to species depending on the amount of lipid storage and growth rates. *Daphnia* has low storage and fast growth compared to other zooplankton such as copepods. That *Daphnia* correlated better with concurrent seston profiles than *Holopedium*, especially with respect to C₁₈ FAs found in cell membranes, indicates faster growth rates, either due to species specific traits or to habitat specialization. That *Daphnia* still exhibits strong correlations with the seston FA composition collected two weeks prior reflects a slower metabolic rate than is seen under lab conditions. *Holopedium* may have a larger lipid storage supply than *Daphnia*, thus evening out immediate effects of diet. It is also possible that the difference in the timing of the correlation between the cladocerans and the seston is an artifact of sampling. *Daphnia* of various life stages were included in the FA analysis, but the *Holopedium* were primarily adults. When cladocerans are growing, their FA pool is not only turning over, but also accumulating, emphasizing the most recently eaten food. The effects of diet on FA in the field are likely to be harder to detect than in diet switching experiments in the lab as the seston composition changes more slowly than the complete change in food sources that can be accomplished in a lab setting.

Eggs can make up a large proportion of cladoceran weight (Green, 1956; Durmont et al., 1975). They are also relatively enriched in FAs compared to the body tissue, especially with respect to LIN, ARA and ω 3 PUFAs (Brett et al., 2009). Resting eggs are even more enriched than subitaneous eggs (Abrusán et al, 2007), which could explain the anomalously high total FA content of the *Daphnia* sampled in mid November. The FA composition of eggs in *Daphnia* reared on single sources of food reflects the food source (Müller-Navarra, 2006). Given that FA

enrichment of eggs helps ensure the survival of neonates released under limiting food conditions (Sperfeld and Wacker 2011), eggs likely accumulate FAs in relation to the food source of the mother, but are not likely to turn over their FA content. Therefore, the type of release of neonates that a zooplankton population employs, either asynchronous or as cohorts, will also have an effect on how closely the populations average FA content reflects that of the concurrent seston. A population that releases neonates asynchronously would more likely consistently reflect concurrent seston than a population that releases as cohorts. *Holopedium* tend to release as synchronous cohorts (Tessier, 1986a) which may also explain why their FA profile best correlated with the seston FA from two weeks prior with respect to LIN and ω 3 PUFAs.

Temperature Correlation with Cladoceran Fatty Acids

Relative and absolute values of SDA in both cladocerans as well as ALA in *Holopedium* only, increased with decreasing temperature. Sperfeld and Wacker (2012) found increased SDA and ALA under cooler conditions in the body tissue of *Daphnia* fed algae rich in these FAs. They, as well as Schlechtriem et al. (2006), found a higher level of EPA in *Daphnia* at low temperatures necessary for successful reproduction and/or maintenance of membrane fluidity. The lack of correlation found in this data set between temperature and EPA indicates a predominant effect of food on this fatty acid, suggesting that EPA was in adequate supply in the seston over the course of this study. It is also possible that the depth-averaged temperature range (7-14°C) in this study was not large enough to show this effect.

It is interesting to note that the relative increase in *Holopedium*'s oleic acid content with temperature does not translate into an absolute increase in oleic acid with temperature, whereas the relationships noted between SDA, ALA, and ARA and temperature are true in relative as well as absolute FA terms. The increase in ARA with temperature in *Holopedium* is much stronger in relative than absolute terms. Increase of a PUFA such as ARA with temperature is in direct opposition of the maintenance of optimal membrane fluidity as described by Hazel (1995), but could be related to the conservation of ARA relative to other FA (Taipale et al. 2011) and increased conversion of LIN to ARA under food stress (Schlechtriem et al. 2006). Alternatively, the increase could demonstrate a larger ratio of egg mass to body mass under warmer conditions as the eggs of cladocerans have been found to be relatively enriched in ARA compared to body tissue (Wacker and Martin-Creuzburg, 2007). Both *Daphnia* and *Holopedium* showed a decrease

in MUFA as temperature increased, contrary to the findings of Sperfeld and Wacker (2012) under conditions of high PUFA food, but in accordance to the experiment of Schlechtriem et al. (2006) with lower PUFA food, perhaps associated with maintenance of optimum membrane fluidity.

Seston Variability Compared to Lake Washington

It has been suggested that the variability in the seston FA values contributes to the ability to use FAs as biomarkers, with higher variability seston values yielding clearer results in the zooplankton. Some fatty acids were more variable in the seston than others; coefficients of variation ranged from 0.20 to 3.26. This is comparable to the range of coefficients of variability in the FA composition of Lake Washington which provided clear FA biomarkers (Ravet et al., 2010). The average coefficient of variability in Lake Washington seston was slightly higher than that in Lake Chaplain, 1.30 compared to 0.92. Especially for *Daphnia*, there is clear co-variation with the seston FA values in this data set, supporting the use of FAs as biomarkers.

Potential Biomarkers

The FAs that exhibit coefficients of variation greater than 0.5 and are also a substantial component of the total FAs are good candidates for biomarkers. FAs that demonstrated this much variation and also comprise over 3% of the seston FA composition on average include 24:1n-7, 18:0, LIN and SDA. In order to function as biomarkers these FAs need to be present in the organisms in question and demonstrate co-variation. Of the above FAs, LIN shows the strongest co-variation with both *Holopedium* and *Daphnia*. 24:1n-7 also co-varied strongly with *Daphnia* and to a lesser extent, *Holopedium*. 18:0 varies with *Daphnia* as well. SDA co-varied weakly with *Holopedium* and *Daphnia*.

No component of the phytoplankton taxa correlated with 24:1n-7 in the seston. It was however, very variable in the seston and made up a large component of the seston and *Daphnia* FAs. Contrary to literature findings (Table 3), LIN correlated positively to the phytoplankton at the taxon level for Pyrrophyta. Both cladocerans and the seston showed strong seasonal patterns in this FA. *Daphnia* showed correlations between LIN and the available biovolume of Pyrrophyta. *Holopedium* did not correlate as well with the seston LIN composition as *Daphnia*, especially mid-summer. SDA was associated with the cryptophytes. *Daphnia* correlated weakly with this FA in the seston as did *Holopedium* at a two week offset. Both cladocerans seem to be

utilizing cryptophytes. SDA also demonstrated a temperature related variation in both the cladocerans. Oleic acid, the major component of the C₁₈ MUFAs was implicated by PCA as a component that explained much of the difference between *Daphnia* and *Holopedium*, could serve as a biomarker. It was a large portion of the seston FA composition. The pattern of association between seston and cladoceran oleic acid composition follows a similar pattern to LIN, with *Daphnia* correlating well during the entire season, and *Holopedium* not following the increase seen in the seston during the mid summer. Oleic acid varied with temperature in *Holopedium* but not in *Daphnia*.

Fatty Acid Content in Comparison to Other Studies

The absolute FA content of *Holopedium* and *Daphnia* in this study was 227±29 and 171±82 (µg FA)(mg C)⁻¹ respectively. In comparison, Persson and Vrede (2006) found 117±26 and 147±63 (µg FA)(mg C)⁻¹ for *Holopedium* and *Daphnia* and Smyntek et al. (2008) found *Daphnia* in the range of 232±56 (µg FA)(mg C)⁻¹ and *Holopedium* as low as 93 (µg FA)(mg C)⁻¹. Smyntek et al. did not find any significant differences between *Holopedium* and *Daphnia* in terms of absolute EPA, DHA, ALA, ARA, and LIN content. In this data set, absolute values for EPA, DHA, ARA, and SDA were all significantly higher in *Holopedium*. Persson and Vrede found no difference in terms of percentage DHA, ARA, SDA, ALA and LIN content, but did find that *Holopedium* had a higher EPA content than *Daphnia*. In contrast, this data set showed differences in the percent composition of DHA, EPA, ARA, SDA, and LIN between *Holopedium* and *Daphnia* with *Holopedium* having higher values for all but LIN. The greater variability in FA composition between the two cladocerans in this study could be related to the larger sample size. It could also be related to differences in food availability and temperature that might mask or highlight differences between the two species.

Fatty Acid/Diet Model

A model based on laboratory examination of *Daphnia* FA profiles and the corresponding profiles of their food (Brett and Martin, unpublished) including cryptophytes, chlorophytes, diatoms and cyanophytes was applied to the FA data from the *Daphnia* and *Holopedium* from Lake Chaplain. The model was able to fit the data with r² values between 0.6 and 0.8. It indicated that *Daphnia* consumed mostly cryptophytes (59% ± 8%) followed by cyanophytes (31% ±

10%) and diatoms ($7\% \pm 5\%$). The model also indicated that *Holopedium* consumed primarily cryptophytes ($71\% \pm 6\%$) followed by diatoms ($23\% \pm 8\%$). In comparison to the biovolume of phytoplankton present in the lake, the model predictions for *Daphnia* cryptophyte consumption was reasonable ($r^2=0.8$). However, for the other phytoplankton taxa examined by the model the predicted consumption ratios were quite different from the actual biomass. This could reflect selective feeding by the cladocerans, which is likely for diatoms and cryptophytes. Alternatively, seston FA sources present in Lake Chaplain that are not included in the model are acting as analogues to the modeled taxa, such as pyrrhophytes with cyanophytes or chrysophytes with diatoms. Input for chrysophytes and pyrrhophytes, both of which are large components of the phytoplankton biovolume in Lake Chaplain, would need to be added to the model in order to make it fully applicable for Lake Chaplain field studies.

Recommendations for Further Work

Should further FA work be conducted, it should be done in conjunction with a GC-MS to aid in peak identification. Unfortunately, during the 2011 season, *Holopedium* never were abundant. It would be interesting to see if the dynamics of *Holopedium*'s FA profile are different under conditions when their population is not under any limitations. It would also be of value to run FA profiles of *Holopedium* with the sheath removed, as colonization of the sheath by ciliates or protozoa could affect the FA profile of *Holopedium*. Updating the model to account for the missing phytoplankton taxa could be beneficial. Sampling the seston FAs above and below the thermocline separately could confirm resource segregation between *Holopedium* and *Daphnia* based on depth. Competition between *Holopedium* and mixotrophic phytoplankton in the epilimnion could be considered. Because of the likelihood for large allochthonous inputs to the lake, it would be useful to have a measure of the magnitude and timing of these inputs.

Conclusions

Holopedium could be well suited to out-compete *Daphnia* at times when phytoplankton such as chrysophytes and diatoms are abundant in the epilimnion, but other phytoplankton such as cryptophytes, are not available. *Holopedium* seems more likely to persist under essential FA poor conditions, positioning them to be able to take advantage of the onset of an essential FA rich phytoplankton bloom. *Daphnia* seem to be more dependent on contemporaneous resources than

Holopedium. Because of their diel migration patterns, *Daphnia* are better able to take advantage of phytoplankton blooms at or below the thermocline, which are common in oligotrophic lakes such as Chaplain. Under stratified condition, *Daphnia* may have access to the best food, whereas mixed conditions or phytoplankton blooms above the thermocline allow both cladocerans access to similar quality food.

During the winter it is too cold and food is too scarce for *Holopedium* to develop an appreciable population. Depending on the spring phytoplankton succession, *Holopedium* may be more able to utilize the available resources than *Daphnia* as food and temperature cease to be limiting. Because of their tendency to stay in the epilimnion, *Holopedium* are likely to be food limited during the times of year when temperature is not limiting, making large summer populations unlikely. The large variation in the timing of the fall population peak of *Holopedium* is likely due to its dependence on either a surface phytoplankton bloom (earlier) or fall turnover (later) as a release from food limitation. The timing, species, and location of phytoplankton blooms as well as the degree of stratification all affect whether *Holopedium* or *Daphnia* is the predominant cladoceran in Lake Chaplain.

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Appendix 1: Fatty Acid Results. Average, standard deviations, correlations and t-test results for concurrent FA profiles and the FA of the seston or *Daphnia* sampled two weeks prior to the *Holopedium*. Significant correlations are bolded. Italicized FA names indicate identification based on retention times provided by Brett or Galloway (2011, personal communication), all other FA identification is based on Supelco 37 Standard or Nucheck 40 Standard

Fatty Acid	Seston			Daphnia			Holopedium			Concurrent						Two Week Offset					
	Average \pm SD	Daphnia Average \pm SD	Holopedium Average \pm SD	Seston/Daphnia		Seston/ Holopedium		Daphnia/ Holopedium		Seston/Daphnia		Seston/ Holopedium		Daphnia/ Holopedium							
				r ²	p	r ²	t-test	r ²	p	r ²	p	r ²	t-test	r ²	p	r ²	t-test				
c14:0	7.0 \pm 1.9	4.6 \pm 1.5	9.4 \pm 1.8	0.02	0.68	0.00	0.03	0.60	0.00	0.11	0.30	0.00	0.25	0.10	0.00	0.03	0.57	0.01	0.00	0.96	0.00
14:1n-5	1.1 \pm 0.3	0.6 \pm 0.2	0.6 \pm 0.7	0.14	0.21	0.00	0.36	0.04	0.01	0.39	0.03	0.79	0.11	0.29	0.00	0.08	0.38	0.04	0.38	0.03	0.79
c15:0	1.1 \pm 0.9	0.5 \pm 0.2	0.7 \pm 0.3	0.26	0.07	0.19	0.20	0.14	0.28	0.24	0.11	0.09	0.41	0.03	0.37	0.09	0.33	0.63	0.48	0.01	0.09
c16:0	17.1 \pm 3.8	16.5 \pm 1.4	13.8 \pm 1.2	0.69	0.00	0.67	0.53	0.01	0.02	0.38	0.03	0.00	0.08	0.37	0.58	0.21	0.14	0.03	0.08	0.38	0.00
16:1n-7t	1.2 \pm 0.6	1.2 \pm 0.3	1.1 \pm 0.1	0.34	0.04	0.51	0.32	0.05	0.87	0.76	0.00	0.13	0.00	0.95	0.33	0.01	0.78	0.60	0.10	0.31	0.13
16:1n-7c	3.9 \pm 1.1	3.3 \pm 0.9	3.2 \pm 0.5	0.21	0.12	0.15	0.10	0.31	0.14	0.04	0.51	0.79	0.00	0.85	0.08	0.05	0.47	0.21	0.49	0.01	0.79
c17:0	0.1 \pm 0.1	0.5 \pm 0.2	0.2 \pm 0.2	0.03	0.59	0.00	0.03	0.60	0.01	0.01	0.79	0.00	0.36	0.04	0.00	0.05	0.46	0.02	0.00	0.87	0.00
c18:0	6.1 \pm 4.4	5.0 \pm 1.3	4.9 \pm 1.1	0.44	0.01	0.41	0.02	0.62	0.28	0.13	0.24	0.55	0.08	0.37	0.38	0.53	0.01	0.19	0.50	0.01	0.55
18:1n-9	9.0 \pm 3.5	12.2 \pm 2.4	5.5 \pm 0.9	0.68	0.00	0.01	0.27	0.08	0.01	0.38	0.03	0.00	0.40	0.03	0.02	0.56	0.01	0.01	0.59	0.00	0.00
18:1n-7	1.6 \pm 0.4	4.6 \pm 0.9	3.2 \pm 0.8	0.00	0.92	0.00	0.07	0.40	0.00	0.01	0.71	0.00	0.10	0.31	0.00	0.00	0.93	0.00	0.15	0.21	0.00
18:2n-6	4.3 \pm 2.5	6.9 \pm 1.9	4.9 \pm 0.9	0.76	0.00	0.01	0.37	0.04	0.41	0.55	0.01	0.01	0.69	0.00	0.01	0.52	0.01	0.53	0.53	0.01	0.01
18:3n-6	0.2 \pm 0.3	0.8 \pm 0.3	0.8 \pm 0.1	0.02	0.61	0.00	0.01	0.77	0.00	0.14	0.22	0.92	0.06	0.46	0.00	0.32	0.05	0.00	0.01	0.73	0.92
18:3n-3	4.0 \pm 1.5	7.3 \pm 1.4	6.4 \pm 1.3	0.36	0.03	0.00	0.12	0.26	0.00	0.59	0.00	0.09	0.35	0.04	0.00	0.46	0.02	0.00	0.50	0.01	0.09
18:4n-3	4.2 \pm 2.4	7.8 \pm 2.5	10.4 \pm 2.7	0.27	0.07	0.00	0.01	0.78	0.00	0.04	0.55	0.00	0.21	0.14	0.00	0.36	0.04	0.00	0.48	0.01	0.00
20:4n-6	0.0 \pm 0.0	3.2 \pm 0.7	6.8 \pm 1.4	-	-	0.00	-	-	0.00	0.01	0.79	0.00	-	-	0.00	-	-	0.00	0.26	0.09	0.00
20:3n-3	0.7 \pm 0.5	0.3 \pm 0.8	0.1 \pm 0.2	0.02	0.66	0.00	0.00	0.96	0.00	0.06	0.45	0.78	0.13	0.26	0.01	0.21	0.14	0.00	0.07	0.40	0.78
20:5n-3	1.8 \pm 1.0	12.7 \pm 1.8	16.3 \pm 1.6	0.35	0.03	0.00	0.20	0.15	0.00	0.03	0.62	0.00	0.06	0.44	0.00	0.38	0.03	0.00	0.03	0.58	0.00
22:1n-9	1.6 \pm 1.5	0.0 \pm 0.1	0.1 \pm 0.2	0.14	0.20	0.00	0.12	0.28	0.00	0.64	0.00	0.41	0.01	0.77	0.00	0.02	0.70	0.00	0.03	0.58	0.41
22:5n-3	2.1 \pm 1.0	0.6 \pm 0.3	1.3 \pm 0.3	0.13	0.23	0.00	0.02	0.64	0.01	0.01	0.78	0.00	0.00	0.93	0.00	0.02	0.63	0.02	0.06	0.43	0.00
24:1n7	6.1 \pm 4.8	3.1 \pm 2.9	1.8 \pm 1.6	0.81	0.00	0.06	0.39	0.03	0.00	0.38	0.03	0.14	0.08	0.37	0.06	0.13	0.25	0.00	0.20	0.14	0.06
SAFA	31.6 \pm 5.8	27.8 \pm 1.6	30.3 \pm 1.6	0.44	0.01	0.04	0.45	0.02	0.49	0.31	0.06	0.00	0.00	0.85	0.03	0.00	0.90	0.45	0.02	0.65	0.00
MUFA	26.4 \pm 2.4	25.0 \pm 2.5	15.7 \pm 2.1	0.33	0.04	0.17	0.06	0.44	0.00	0.25	0.10	0.00	0.00	0.83	0.14	0.17	0.18	0.00	0.24	0.11	0.00
ω 6 PUFA	4.5 \pm 2.5	11.0 \pm 1.6	12.7 \pm 2.0	0.52	0.01	0.00	0.28	0.07	0.00	0.52	0.01	0.04	0.66	0.00	0.00	0.58	0.00	0.00	0.53	0.01	0.08
ω 3 PUFA	13.9 \pm 5.2	30.7 \pm 4.2	36.4 \pm 4.2	0.23	0.09	0.00	0.14	0.23	0.00	0.25	0.10	0.00	0.22	0.13	0.00	0.50	0.01	0.00	0.53	0.01	0.00
Unidentified	26.5 \pm 7.5	7.4 \pm 2.0	7.5 \pm 2.4	0.30	0.05	0.00	0.07	0.40	0.00	0.01	0.81	1.00	0.00	0.92	0.00	0.05	0.49	0.00	0.02	0.67	0.93
ω 3/ ω 6	3.8 \pm 2.2	2.9 \pm 0.7	3.0 \pm 0.7	0.60	0.00	0.39	0.38	0.03	0.59	0.68	0.00	0.64	0.73	0.00	0.56	0.64	0.00	0.82	0.73	0.00	0.44