Habitat Selection and Ecological Stoichiometry:
The Role of Seston C:P in *Daphnia* Daytime Spatial Location

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ABSTRACT

Diel vertical migration (DVM) of zooplankton has intrigued ecologists for over 100 years. Traditional DVM theory holds that *Daphnia* migrate into the hypolimnion during the daytime to escape predation pressure from visually-hunting predators, while nighttime ascent into the epilimnion facilitates feeding and growth. It has been demonstrated that *Daphnia* behavior may be altered by algal quantity, predator cues, and temperature or light levels. However, little was known about how algal quality (in terms of nutrient content) affects *Daphnia* vertical position. This dissertation examines the effects of algal quality on the daytime habitat selection of *Daphnia* through laboratory experiments and analysis of field data.

Laboratory studies examined the movements of *Daphnia* in a thermally-stratified water column. When algal quality was high (C:P ~ 150) throughout the water column, mature *Daphnia* were found in the epilimnion during the daytime, despite the presence of a predator cue. However, when algal quality was low (C:P ~1500) throughout the water column, mature *Daphnia* tended to spend the daytime in the hypolimnion. These habitat preferences were not detected when the experiment was repeated using young juveniles (age 0-5 days), though at age 6 days, juveniles' behavior appeared to begin to shift toward that of adults. As expected, body mass, fecundity, and net reproductive rate were found to be positively correlated with increased environmental temperature, and fecundity and net reproductive rate were positively correlated with increased food quality.

A field study of 34 lakes revealed that habitat selection was not only food-quality dependent, but also species-specific. *D. retrocurva* and *D. mendotae* exhibited a preference for the upper lake layer when food quality was good throughout the water column, but not when food quality was poor in the upper layer. *D. pulicaria* did not exhibit any layer preference in either lake environment.

The results from this research provide insight into *Daphnia*'s ability to balance the multiple factors associated with a heterogeneous vertical gradient in order to realize maximum productivity, and thus maximum fitness, in the presence of visually-hunting predators. The results further indicate that food quality is an important factor in determining habitat selection, productivity, and ultimately fitness.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 2: What's P got to do with it? The effects of food quality on *Daphnia* vertical position

Chapter 3: Effects of food quality and temperature on juvenile *Daphnia* behavior and development

Chapter 1

Introduction and literature review: A brief history of the study of vertical migration and the importance of considering ecological stoichiometry

Introduction

Understanding the relationships and dynamics between zooplankton and their environment is a major focus of many limnological studies. In many lakes, zooplankton exhibit diel vertical migration patterns, spending the nighttime in the warmer, upper waters of the epilimnion and the daytime in the cooler, lower waters of the hypolimnion. It has been repeatedly demonstrated, and is generally well-accepted, that the ultimate cause of zooplankton migration is to reduce the predation pressure they face from visually-hunting planktivores (Zaret and Suffern 1976; Gliwicz 1986; Ohman 1990, Bollens and Frost 1991; Bollens and Stearns 1992). However, when migrating through a vertical column of water, zooplankton encounter a range of conditions including variations in light (including UV), temperature, dissolved oxygen, and food quantity and quality. While variations in these environmental conditions are unlikely to be the ultimate cause of vertical migration, these proximate issues are of potential importance to zooplankton ecology.

It is well known that algal abundance is not distributed homogeneously throughout the water column (Orcutt and Porter 1983; Loose 1993). Algal quality (defined throughout this dissertation as the atomic ratio of carbon to phosphorus, though also in terms of size, digestibility, and amount of lipids) is also not homogeneously distributed. While previous research assumed the epilimnion to be very food-rich (McLaren 1974; Zaret and Suffern 1976; Enright 1977; Stich and Lampert 1981; Guisande et al. 1991), it has since been argued that epilimnetic algae is often of lower nutritive quality, and that algal quality might be better in deeper waters (Williamson et al. 1996; Sterner et al. 1997; Sterner et al. 1998). In contrast, the epilimnion, with its abundant light but fewer nutrients, produces more carbon-rich, lower quality food.

In addition to variations in algal quality and quantity experienced when migrating through a stratified lake, zooplankton also encounter a range of temperatures. Researchers have shown low temperatures to have negative effects on zooplankton growth rate (McLaren 1963; Sterner et al. in prep). Therefore, it has been argued that a zooplankton's ascent out of deep water may in part be to experience the warm temperatures in the epilimnion and thus to increase metabolic activity. Thus, throughout the diel cycle, zooplankton experience trade-offs among numerous factors including predation pressure, algal quantity and quality, and temperature (Lampert et al. 2003).

Because recent research has demonstrated that the epilimnion may not contain the most nutrient-rich algae after all, migration may be even more of a trade-off than previously believed. The epilimnion, with its warmer temperatures, benefit zooplankton by facilitating a faster growth rate; however, the cooler hypolimnion offers protection from predators and potentially higher quality food. Perhaps a balance between temperatures and food conditions (both quality and quantity) might determine where zooplankton should be distributed in the daytime when they perform diel vertical migrations (DVM). Additionally, evidence may be provided to show that a balance among the multiple factors associated with the vertical gradient could help zooplankton realize maximum productivity, and thus maximum fitness, in the presence of visually-hunting predators. The intent of this study was to investigate this trade-off and to merge the studies of vertical migration and zooplankton nutrition in order to broaden the understanding of plankton ecology. Specifically, the following hypotheses were tested:

- Zooplankton are able to react behaviorally to variations in the nutritional quality of their environment. (Chapter 2)
- Growth rate and fecundity are affected by a zooplankter's vertical migration through layers of nutritionally varied water. (Chapter 3)
- Zooplankton daytime behavior from a large-scale sampling effort was similar to daytime behavior in a model system in the laboratory. (Chapter 4)

In addition, we also created a food quality/vertical migration mathematical model to explain why *Daphnia* may risk predation and forego vertical migration in order to remain in the epilimnion at all times.

I found that, as adults, zooplankton are indeed able to react behaviorally to variations in seston quality. When presented with low quality food, animals remained lower in the water column during the daytime compared with animals presented higher quality food. More interestingly, there was an interaction between the presence/absence of fish kairomone and food quality. When *Daphnia* were presented with a predator cue, the difference in their behavior was more pronounced than when their environment was kairomone-free. Thus, the evolution of vertical migration in adult *Daphnia* might not be as simple as merely predator avoidance.

While adult *Daphnia* exhibited varying patterns in their daytime behavior depending on presence/absence of kairomone and algal quality, juvenile *Daphnia* did not exhibit this same phenomenon. Young (and therefore smaller sized) animals tended to remain fairly high in the water column throughout the daytime, however, as the juveniles matured and became larger, they began to exhibit the same divergent behavior patterns as the adults.

Growth rate and fecundity were both affected by the environment. Animals fed high quality food in a warm environment grew larger faster than did animals that were fed high quality food in a cool environment. The same pattern was exhibited by animals fed low quality food in warm and cool environments. There was a significant effect of location of animals (cool vs. warm temperatures) in determining their growth rate, but there was not a food quality effect.

Dispersal of *Daphnia* from a field study was similar to daytime depths of laboratory animals. When food quality was good throughout the entire water column, *Daphnia* appeared to attempt to maximize their fitness by choosing the layer that would offer the fastest growth rate (the upper layer). In contrast, when food quality was good in the lower layer and poor in the upper layer, no significant lake-layer density difference was noted among the *Daphnia* species. Interestingly, *D. retrocurva* and *D. mendotae* lake-layer preference differed depending on the nutrient profile of the lake; *D. pulicaria* did not exhibit any layer preference regardless of the nutrient status of the lake.

This project thus broadens the understanding of vertical migration, a field that has been investigated for more than a century. The results from this study also provide valuable insight into the study of ecological stoichiometry, a field of ecology that examines the balance of multiple chemical elements in ecological interactions (Sterner and Elser 2002). Finally, this study lays the foundation for further examining the degree of homeostasis, the resistance of change of internal conditions despite variability in the external environment, in vertically migrating *Daphnia*.

LITERATURE REVIEW

Vertical migration

It has long been known that many taxa of zooplankton exhibit diel vertical migrations, spending the daytime in the hypolimnion of a lake and the nighttime in the epilimnion. Migration amplitudes have been recorded from a few meters to over 100 meters (Hutchinson 1967). According to Lampert (1989), the presence of vertical migration in so many zooplankton taxa suggests that there is some sort of an adaptive value in the migration for the animals. Several hypotheses regarding the ultimate reason for migration have been formulated, tested, and debated in the literature. The two main hypotheses which attempt to mechanistically explain the ultimate presence of this phenomenon are (1) zooplankton migrate in order to gain a metabolic advantage and (2) zooplankton migrate in order to decrease their mortality risks.

Ultimate reasons for zooplankton DVM:

Migration to increase individual productivity

McLaren (1963, 1974) proposed the idea that vertical migration may be adaptive to zooplankton by giving migrators a metabolic advantage over nonmigrators. According to him, when the surface waters of a lake are warm and the lower layers are cool, animals could achieve an energetic bonus by resting in the cold hypolimnion during the day and then ascending to feed in the warm waters at nighttime. When spending time in the cooler waters of the hypolimnion, animal growth rate is slowed. McLaren proposed that this growth retardation is advantageous to zooplankton and results in higher fecundity. In his laboratory studies, he found that copepods that were grown at a lower temperature reached a larger adult body size. He assumed that under non-limiting food conditions larger animals experienced increased fecundity. Therefore, zooplankton achieved not only a metabolic advantage by migrating, but also a demographic advantage (Lampert 1989). Laboratory studies of this hypothesis have contested this idea. In his studies on

Chaoborus, Swift (1976) determined that there is no energetic bonus for migrators. Moreover, Stich and Lampert (1984) performed an experiment in which coexisting species of *Daphnia* from Lake Constance (Germany) were placed in flow-through chambers and exposed to either fluctuating or constant temperature and food gradients. They found that both species grew faster and produced more offspring when in the constant or nonmigrating conditions. Thus, they, too, demonstrated there to be no metabolic advantage for vertical migration.

Enright (1977) elaborated upon McLaren's metabolic hypothesis by incorporating two assumptions. First, he assumed that algal quantity and quality are highest at dusk since photosynthesis occurs during the day and there is an increase in respiration and grazing at nighttime. He also assumed that since the algal quantity is low in the morning, zooplankton retreat to the colder hypolimnion to respire. At nighttime, they return to the upper waters and feed at an increased rate in order to compensate for the daytime starvation in the hypolimnion. Thus, as McLaren hypothesized, zooplankton may gain an energetic profit by migrating. While McLaren was unable to offer an explicit reason why zooplankton migrated at the times of day that they did, Enright's model offered an explanation. While this hypothesis made intuitive sense for some animals, a series of experiments found that this phenomenon was not applicable to certain zooplankton, such as the easily-satiated *Daphnia* (Lampert et al. 1988). In addition, recent studies have shown that algal biomass is not necessarily of higher quality when it has been left ungrazed. Intense grazing pressure may produce higher quality food (McNaughton 1988; Sommer 1992; Urabe 1995; Makino et al. 2002).

Geller (1986) disagreed with Enright's "energetic profit" theory and argued that zooplankton do not always migrate in lakes where food is abundant. Specifically, he noted that in Lake Constance *Daphnia hyalina* do not migrate in the spring when food is abundant but instead migrate later in the summer when food is limiting. He hypothesized that *D. hyalina* populations remained near the surface of the lake during the spring and constantly fed in order to use an "exploitative" strategy and build up its population. However, as summer approached, *D. hyalina* switched to a "conservative" strategy and began to migrate. He titled his hypothesis the "starvation avoidance hypothesis." He proposed that in the late summer, food abundance is unpredictable, so periods of starvation may occur. Since energy losses of animals in the warm epilimnion are higher than in the cooler hypolimnion, it would be beneficial to reduce energy losses as much as possible. He applied a mechanism known to occur in marine snails (Somero and Hochachka 1976) in which the animals divided their time between cold and warmer water. When the snails moved to warmer waters they rapidly increased their feeding rate but their respiration rate did not increase as rapidly. They could then return to the cooler waters to digest before there was a large increase in their respiration rate. Therefore, they "gained energy" in order to compensate for the starvation losses. This effect, however, has not been demonstrated to occur in zooplankton in the field or the laboratory (Lampert 1989).

Migration to decrease mortality

The second group of hypotheses to explain the ultimate reason for DVM emphasizes vertical migration as a survival mechanism. Instead of gaining an energetic or metabolic bonus or increasing fecundity, this hypothesis states that zooplankton migrate in order to decrease mortality.

One way zooplankton may reduce mortality is to migrate in order to escape the deleterious effects of UV light (Hairston 1976; Siebeck 1978 as cited in Lampert 1989; Hanazato et al. 2002; Winder and Spaak 2002; Ringelberg and Van Gool 2003; Cooke et al. 2008). This hypothesis suggests that despite the partial filtering effects of water, daytime exposure to sunlight could produce physiological damage to migrating organisms. While this hypothesis may explain migrations of a few meters, it would most certainly not explain observed deep migrations as UV light is absorbed in the upper few meters of the water column (Lampert 1989; Ringelberg 1999).

The most widely supported rationale for the existence of vertical migration behaviors is that zooplankton minimize their mortality from predation by migrating away from their predators. Zaret and Suffern (1976) were the first to publish the theory that zooplankton descend in the daytime in order to escape visually-hunting predators (Lampert 1989). The well-lit epilimnetic environment provides no hiding places for zooplankton to escape visual predators. Further studies have shown that the zooplankton taxa most likely to migrate include those lacking an effective escape response (Drenner et al. 1978; Wright and O'Brien 1984) and larger zooplankton with pigments or eggs, which are easier for visually-hunting predators to see (Wright et al. 1980).

It has been hypothesized that the amplitude of migration should vary in accordance with the magnitude of predation pressure, abundance of planktivorous fish or invertebrates (Stich and Lampert 1981; Dodson 1988), and in relation to the clarity of the water column (Dodson 1990). Thus the costs of vertical migration (retardation in growth rates: Orcutt and Porter 1983; Dawidowicz and Loose 1992a) are offset by the benefits of reduced predation (Ohman 1990; Bollens and Frost 1991; Bollens and Stearns 1992). Gliwicz (1986) provided strong evidence that migration is tied to predators in a study he performed in lakes of the Tatra mountains. While there was no migration in fishless lakes, there were very strong migration patterns in lakes that had contained fish for over 1000 years. In addition, in laboratory (Leibold 1990; Ringelberg 1991; Loose et al. 1993; Dawidowicz et al. 1990; DeMeester 1993) and field (Bollens and Frost 1989; Dini and Carpenter 1992; Nesbitt et al. 1996) experiments, the addition of fish kairomones has caused previously nonmigratory zooplankton to begin to migrate through the water column.

Environmental variables affecting zooplankton DVM:

As mentioned, the majority of researchers conclude that the ultimate reason for zooplankton migration is avoidance of visually-hunting predators. In their vertical migrations, however, zooplankton encounter a range of physical and chemical conditions including changes in temperature, light, and seston quantity and quality. While studies have examined how temperature, light and algal quantity gradients affect *Daphnia* (Ringelberg 1964; Daan and Ringelberg 1969; Lock and McLaren 1970; Kirby 1971; Halbach 1973; Keen 1979; Orcutt and Porter 1983; Pijanowska and Dawidowicz 1987; Johnsen and Jakobsen 1987; Gliwicz and Pijanowska 1988; Forward 1988; Haney et al. 1990; Ringelberg et al. 1991; Forward and Hettler 1992; Makino et al. 1996), few studies have attempted to understand the implications of algal quality gradients on vertically migrating zooplankton (but see Schatz and McCauley 2007: Reichwaldt 2008).

Effects of the light/dark cycle:

Changes in light intensity have long been considered to be crucial to *Daphnia* migration and have been argued to act as a controlling, initiating, and orienting cue during migration (Forward 1988). Understanding exactly how zooplankton are able to react to changes in light attenuation is a much more complex matter. Ringelberg proposed that relative changes in light intensity and phototaxis are a primary initiating cause and mechanism for vertical migration (Ringelberg 1964; Daan and Ringelberg 1969) and correlated maximum relative vertical position change with light intensity change in the field (Ringelberg et al. 1991). In a four-year study, he correlated relative light intensity and predator presence with vertical migrations of *Daphnia galeata x hyalina* in Lake Maarsseveen, Amsterdam (Ringelberg and Flik 1994). Numerous studies have concurred with Ringelberg's assessment that changes in light intensity are a predominant factor that cause zooplankton to begin their migration through the water column (Haney et al. 1990; Forward 1988; Forward and Hettler 1992; Makino et al. 1996; Fortier et al. 2001; Van Gool and Ringelberg 2003).

Another factor that alters the light intensity in a water column is the concentration of dissolved organic carbon. As DOC concentrations increase, water becomes more colored, thus offering some protection from visually-hunting predators. Therefore zooplankton in highly stained lakes may exhibit less dramatic DVM patterns than in those lakes that have a greater Secchi depth (Wissel and Ramacharan 2003; Dejen et al. 2004).

Effects of temperature:

Multiple studies have been performed in order to determine the effects of warm versus varying temperature on zooplankton life history parameters (Lock and McLaren 1970; Kirby 1971; Halbach 1973; Keen 1979). These studies have concluded that zooplankton growth rate is highest in warm temperatures. McLaren's "energy bonus" hypotheses (1963, 1974) proposed that even though they may not grow as rapidly at cooler temperatures, zooplankton reach larger adult body size and have enhanced fecundity. However, Orcutt and Porter (1983) found that when food was not limiting, mature body size and longevity of *Daphnia* were insensitive to experimental temperature differences. Brood size, brood number, total young per reproductive female and net reproduction rate were found to be highest at a midrange temperature. In addition, they found that age at onset of reproduction, instar at first reproduction and brood duration decreased with increasing temperature. When integrating the patterns of survivorship and fecundity over time in order to indicate fitness, they found no thermal demographic advantage to migration and predicted maximum fitness when zooplankton remained in warmer surface waters.

Vertical distributions of zooplankton can also differ depending on age and size classes of the organisms. Kessler and Lampert (2004) found that temperature was the strongest factor governing the distribution of *Daphnia* in a large-scale mesocosm. They found that egg-bearing females were the least likely to remain in the cooler hypolimnion during the nighttime, presumably in order to increase both the mean temperature experienced and development rate. In addition, the shallower the temperature gradient between the epilimnion and the hypolimnion, the more homogeneous the *Daphnia* distribution at nighttime (Lampert et al. 2003; Kessler and Lampert 2004).

Effects of algal quantity:

Numerous researchers have examined algal availability throughout the water column, and there is a widespread assumption that its quantity decreases with depth (MacKay 1996). Depending on the system, however, the magnitude of decrease varies, with estimates ranging from a 100-fold decrease with depth (Orcutt and Porter 1983) to merely a 2-fold decrease (Loose 1993). These food gradients can greatly affect migration patterns. In a review of zooplankton migration literature, Lampert (1989) summed up the results of food availability studies with a three-point model. He noted that vertical migration required energetic costs and that there would be a trade-off to acquire a balance between maximum energy input and maximum protection. Thus, he stated that migration patterns would be expected to change according to algal abundance. Specifically:

- Zooplankton will not migrate if food availability is high in the hypolimnion (Pijanowska and Dawidowicz 1987; Gliwicz and Pijanowska 1988) as there is no benefit under these circumstances to risk increased predation in the upper waters during the day, nor is there the need to waste energy migrating if zooplankton can maintain their growth rate in the hypolimnion.
- With increasingly unfavorable hypolimnetic conditions (such as a decrease in food quantity), zooplankton will gradually begin to spend a larger proportion of the day in the epilimnion, despite the risk of predators.
- When food availability is extremely poor in the hypolimnion, zooplankton can not ingest enough food in the epilimnion during the nighttime to survive in the food-scarce hypolimnion during the daytime. Animals must therefore remain in the epilimnion at all times despite the risk of predation (Johnsen and Jakobsen 1987).

Ecological stoichiometry and vertical migration

Ecological stoichiometry

In simple terms, stoichiometry is the balance of elements. When considering food quality, for instance, one can talk about the amount of phosphorus in the food, but the *relative amount* of phosphorus in algae will change depending on the amount of, for example, carbon, in the algal cells. Many studies have examined the role of stoichiometry in the ecology and evolution of various organisms (e.g. Kay et al. 2005), as a framework for describing population and community ecology (Andersen et al. 2004; Grover 2004; Hille Ris Lambers 2004; Moe et al. 2005), and a unifying concept for examining ecosystems (e.g. Schade e. al. 2005) Thus in referring to the C:P ratio of the food, and in describing the balance of elements in ecological processes, one can, in this case, better understand the nutritional quality of the food source.

Effects of algal quality:

Recently a great deal of research has been published about the effects of food quality on zooplankton life history parameters. Studies have shown that while zooplankton are generally not food limited (above subsistence carbon values) (reviewed by Sterner and Schulz 1998), elemental limitation (especially phosphorus) may limit growth rate and fecundity (Hessen 1990; Hessen 1992; Urabe and Watanabe 1992; Sterner 1993; Sterner and Robinson 1994; Elser et al. 1996; Urabe et al. 1997; DeMott

1998; Schulz and Sterner 1999; Sterner et al. in prep). Despite the numerous nutrition experiments, the majority of these studies have examined zooplankton life history parameters under constant food sources. Since few, if any, zooplankton inhabit a homogeneous environment, these studies do not accurately describe typical lake conditions. In order to understand zooplankton responses to changes in algal qualities, the fields of nutrition and vertical migration must be integrated.

MacKay (1996) was among the first to integrate the studies of zooplankton nutrition and migration. He performed a unique growth and fecundity experiment in order to separate the effects of seston quality from temperature gradient effects. In the laboratory, containers of hypolimnetic and epilimnetic water were incubated at both warm and cool temperatures; in addition he simulated vertical migration in two additional containers by fluctuating their temperatures at dusk and dawn. He found that the physical conditions (specifically the temperature) significantly affected *Daphnia* growth rate and fecundity but that seston source did not affect these parameters. He noted, however, that the seston C:P vertical gradient in Lake 110 was not as steep that year as it had been in previous years and advocated continued research to determine the effects of an increased quality gradient on migrating populations.

Williamson et al. (1996) found algal quality to be maximal in the metalimnion of Lake Wayneswood, Pennsylvania and suggested that the previously held beliefs that epilimnetic food quality is high may not always be true. Additionally, they proposed that because the classic DVM paradigm attempts to explain why zooplankton migrate down out of food-rich surface waters, it should be abandoned.

Sterner and Schwalbach (2001) published another nutrition study that has contributed to the understanding of DVM. Their study examined the effects of varying C:P content on *Daphnia magna* growth rate and showed that animals exposed to high quality algae for less than half the day were able to grow near maximal rates they achieved when they spent the entire day in high quality food. By simulating varying algal quality throughout the water column, this study demonstrated that *Daphnia* may store some of this necessary phosphorous, a sort of "luxury consumption" which allows animals to continue to grow at maximal rates even in the presence of lower quality algae.

Recently, Schatz and McCauley (2007) and Reichwaldt (2008) published studies examining the effects of varying food quality on zooplankton spatial location. Schatz and McCauley examined whether juvenile and adult *Daphnia* were able to detect patches of high quality food within a plexiglass chamber of algae with varying C:P ratios. They found that, when in a heterogeneous environment, both the juveniles and adults were quickly able to locate the high-quality patches. When placed in a homogeneous food environment, foraging appeared to be random.

Reichwaldt further demonstrated that food quality affects *Daphnia* habitat selection through experiments performed in the "plankton towers" (multi-story water columns) at the Max Planck Institute in Plön, Germany. Using two different species of algae (*Scenedesmus obliquus* – considered a high quality algae and *Synechococcus elongatus* – considered a low quality algae), Reichwaldt examined the spatial location of juvenile and adult *Daphnia pulicaria* in a vertically heterogeneous environment. She found that food quality affected adult habitat selection, but that it did not affect habitat selection by juveniles.

Despite an increasing interest in the integration of zooplankton nutrition and vertical migration, there are still large gaps in the knowledge. The present study attempts to discern how vertical migration through horizontal strata of water affect zooplankton behavior, growth, and fecundity.

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Chapter 2

What's P got to do with it? The effects of food quality on adult *Daphnia* vertical position

Abstract

These experiments demonstrated that the daytime location of *Daphnia* depends on food quality and the location of the food and secondarily on the presence or absence of fish kairomone. We examined *Daphnia* fed high-quality (C:P ~ 150) and low-quality (C:P ~ 1500) algae at two different locations (surface or mid-column) in a one-meter deep, temperature-stratified aquarium in order to determine how resource availability and temperature affected *Daphnia* daytime location. We found that *Daphnia* exhibited strong diel vertical migration (DVM), spending the daytime in cooler waters (with *Daphnia* aggregating around 14.5˚C), when fed low quality food. This finding was irrespective of the presence or absence of fish kairomone or of food delivery location. When fed high quality food, *Daphnia* ceased migration, and they remained near the surface of the water (aggregating at around 17.6˚C – again, irrespective of the presence or absence of fish kairomone or food delivery location) during the daytime. This indicates that *Daphnia*

may be able to detect variations in the nutritional content of their food, and they may alter their vertical position in response to varying phosphorus content regardless of whether predators are present or absent dependent upon predator cue presence or absence, water temperature, and food quality. The model shows that DVM is evolutionarily favorable when food quality in the epilimnion is low and when predators are present. We showed that it is evolutionarily favorable for *Daphnia* to remain in warm surface water during the daytime when the gain in growth from being in the warm epilimnion is greater than the increase in mortality experienced in that layer during the day. This study provides unique insight into *Daphnia* habitat selection and ecological stoichiometry.

Introduction

Diel vertical migration (DVM) has been an area of study for well over a century. For the past two decades much of the vertical migration literature has focused on the roles of light (Van Gool and Ringelberg, 2003; Ringelberg 1999; Winder and Spaak 2002; Hanazato et al. 2002), predators (Dawidowicz et al. 1990), and food quantity, (Pijanowska and Dawidowicz 1987; Gliwicz and Pijanowska 1988) in determining zooplankton behavior. At the same time, there has been an increase in understanding of the role of nutrients (specifically phosphorus) for both algae and zooplankton in determining zooplankton growth and community structure (see Sterner and Schultz 1998; Sterner and Elser 2002). Recent studies have focused on the growth rate and egg production of zooplankton under a variety of temperature, food quantity, and food quality regimes (Sterner and Schwalbach 2001; Sterner et al. in prep). Few studies, however, have considered the role of food quality in DVM (but see Schatz and McCauley 2007: Reichwaldt 2008).

Traditional views of vertical migration state that zooplankton tend to spend the majority of the daytime in the hypolimnion in order to avoid visually-hunting predators. A cost of inhabiting this cold lower layer is retardation in growth and delay in egg production (Sterner et al. in prep.). At nighttime, zooplankton may ascend into the warmer waters of the epilimnion, perhaps both to reap the growth benefits of the warmer water and to feed on the normally more abundant algae. Winder et al. (2003) found that even if the epilimnion contains poorer quality algae than the lower layers, *Daphnia* still tend to migrate into this upper layer. It is generally accepted that this behavior is triggered by changes in light intensity and is modified (in terms of timing and/or intensity) by chemical cues from predators (fish kairomone) (Ringelberg 1991; Loose and Dawidowicz 1994; Kessler and Lampert 2004).

This "normal" migratory pattern can also be altered by the presence of predator cues. The presence of invertebrate predators frequently produces a "reverse migration" (Gilbert and Hampton 2001) where zooplankton spend the daytime in the epilimnion and the nighttime in the hypolimnion. The invertebrate predators (usually *Mysis* or *Chaoborus*) "normally" migrate in order to avoid vertebrate predators and the zooplankton reverse migrate in order to avoid the invertebrate predators (Dawidowicz et al. 1990; Lagergren et al. 2008).

Studies have demonstrated that zooplankton also alter their daytime location based on the amount of food present (Johnsen and Jakobsen 1987), water clarity (Wissel and Ramacharan 2003), presence of ultraviolet light (e.g. Hanazato et al. 2002) and ontogeny (e.g. Kessler and Lampert 2004; Lagergren et al. 2008). Lakes are patchy environments and offer a variety of habitats within a column of water. If zooplankton are able to select their environment based on these varying cues, then they might also migrate when there is patchiness in food quality. In this study, I examined *Daphnia* daytime habitat choices in a thermally-stratified water column under conditions of varying temperature, algal P content, and fish kairomone. In addition, a model was created in order to better understand when and why zooplankton may risk predation in order to maximize growth rate.

Methods

The plankton organ

A plankton organ (Fig. 1a) was constructed in order to simulate a stratified lake environment. The basic design of the apparatus was similar to Dawidowicz and Loose (1992a, 1992b). The tank was 1.55 m in length, 0.93 m in height and 0.24 m in depth and was kept in an isolated room in order to minimize unintended environmental variations. In order to visually determine *Daphnia* position, the tank was divided into five horizontal sections numbered one through five (top = 1, bottom = 5) (Fig. 1b). These sections were outlined with red string on the outside of the tank so that section markings would not impede zooplankton movement.

Forty-eight removable plexiglass tubes were hung in the plankton organ to allow manipulation of the food environments for the zooplankton. All tubes were open at the top and were covered with 150 μm Nitex® mesh at the bottom. Solutions such as media, algae, and "fish water" were pumped in at the top, allowed to flow through the tube, and exit through the mesh. The volume within each plankton organ tube remained constant. The introduction of food and media into the top of each plankton organ tube caused the media within the tube to be forced out the bottom tube mesh and into the plankton organ water bath. Thus the algae within each tube both settled naturally and were further pushed out the tube bottom through the introduction of fresh algae and media.

A hollow steel tube, located at the bottom of the tank, was connected to a chiller to cool and regulate temperature in the "hypolimnion." A peristaltic pump was used to mix the water in the hypolimnion and maintain temperature stratification.

A light bank consisting of two 40-watt cool white fluorescent tubes hanging 0.5 meters above the tank provided light. The lights were on an 11:13 hour day/night schedule and were shielded using black paper to ensure proper directionality (minimize scattering) (Fig. 1a). The light levels averaged 13.0 $\mu mol/m^2/sec$ and approximately 6.9 $\mu mol/m^2/sec$ at the surface and bottom of the tank respectively (see Appendix A for other light measurements). In addition, a 4-watt nightlight with a LED sensor approximately 20 cm from the surface of the water was used at nighttime to simulate directional nighttime light. I found this night light to be necessary in order for *Daphnia* to rise to the surface of the plankton organ during the nighttime.

In pilot studies of zooplankton migration, a red light bulb was used in order to observe *Daphnia* during the nighttime. However, even though much has been written about the insensitivity of zooplankton to red light (Novales Flamarique and Browman 2000), obvious changes in zooplankton behavior, such as "circling" and rapid decent toward the bottom of the tank, were noted when this light was used. Hence, a single nighttime observation was performed at the conclusion of this experiment. In all treatments, 95% of the *Daphnia* were at the surface during this nighttime observation. For more specifics about the plankton organ set-up, refer to Appendix A.

Experimental design and data analysis

The *D. magna* used in these experiments were a clone from the Großer Binnensee, a brackish lake in Northern Germany that contains dense populations of fish (Lampert 1991). This *Daphnia* population has been maintained in long-term cultures at the Max Planck Institute for Limnology, and at the University of Warsaw, Poland (Loose and Dawidowicz 1994). Three mature *Daphnia* from a single cohort were transferred into each of 16 plankton organ tubes; mature *Daphnia* were used rather than juveniles, because adults have been reported to have a stronger migratory pattern (Zaret 1980). Tubes were randomized and assigned to one of eight treatments. A 2 x 2 x 2 factorial design was created with two algal qualities (high C:P and low C:P), two predator treatments (fish kairomone present, no kairomone) and two resource locations (eplimnion, metalimnion) and resulted in the following eight treatments: 1) high quality algae in the presence of fish kairomone and with algae introduced at the epilimnion (HT+); 2) high quality algae in the presence of fish kairomone and with algae introduced at the metalimnion (HM+); 3) high quality algae in the absence of fish kairomone and with algae introduced at the epilimnion (HT0); 4) high quality algae in the absence of fish kairomone and with algae introduced at the metalimnion (HM0); 5) low quality algae in the presence of fish kairomone and with algae introduced at the epilimnion (LT+); 6) low quality algae in the presence of fish kairomone and with algae introduced at the metalimnion (HM+); 7) low quality algae in the absence of fish kairomone and with algae introduced at the epilimnion (LT0); and 8) low quality algae in the absence of fish kairomone and with algae introduced at the metalimnion (LM0).

Low (LOP, C:P ~1500) or high (HIP, C:P ~ 150) qualities of the green alga *Scenedesmus obliquus* were cultured in laboratory chemostats and were produced by adjusting the C: N: P ratio of the influent medium and the turnover rate of the chemostat reaction vessels (Urabe et al. 1997). The protocol to create these algal cultures and details on the composition of these cells can be found in Sterner (1993), Sterner and Smith (1993), and Sterner et al. (1993). All algae were added to 3μm filtered lake water (Lake Calhoun, Minneapolis, MN, USA) and was provided in abundance (> 1mg C/l). Drip rates were set to replace the entire volume of each tube twice per day.

Numerous studies indicate that fish kairomone is necessary to stimulate *Daphnia* to migrate (ex. Leibold 1990; Loose et al. 1993). Therefore, in order to add fish kairomone to half of the treatments, 2 goldfish (*Carassius auratus*, each 6 cm long) were added to the treatment water that was dripped into "kairomone +" experimental tubes. In addition, fish were fed approximately 10 *D. magna* clones in order to introduce any chemical distress cues that the *Daphnia* may emit (P. Dawidowicz, pers. comm.). Thus, half the tubes received algae in kairomone-infused lake water and half received algae in kairomone-free lake water. It was assumed that the kairomone was distributed throughout the tubes; however, there was no way to know the precise distribution of kairomone throughout the tubes, because the composition of chemicals that make up this environmental cue is not fully understood. Each day, the reservoirs containing inflow lake water were washed in hot water and refilled with fresh water and algae. This daily washing minimized the build-up of bacteria, which is known to degrade kairomone, in the reservoirs (P. Dawidowicz pers. comm.).

At the beginning of the experiment, each tube was filled with filtered lake water and 1mg C/l of the appropriate food. *Daphnia* were allowed approximately 12 hours to acclimate to the plankton organ tube before location observations began. *Daphnia* vertical position was observed and recorded approximately three times during each day. The experiment was run for four days and then repeated with another *Daphnia* cohort.

To avoid pseudoreplication, all *Daphnia* locations within a tube were averaged so that there was a single depth point for each observation. These daytime tube averages were analyzed using repeated measures ANOVAs in SAS (version 8.0) and Tukey's HSDs in Jmp (version 5.1.2).

Results

During a pilot study, *Daphnia* exhibited classical diel vertical migratory behavior; animals remained near the bottom of the plankton organ during the day and rose to the surface at night (Fig. 2). Although much has been written about *Daphnia* insensitivity to red light, *Daphnia* responded dramatically when exposed to red light during nighttime observations; therefore nighttime observations were discontinued.

Animals fed high quality food, regardless of drip location, remained higher in the water column during the daytime than animals fed low quality food (Fig. 3). This was highly significant ($p < 0.001$) in both experiments (Tables 1 and 2). In the first replicate, animals fed high quality food aggregated at an average temperature of 16.5°C, while animals fed low quality food aggregated at 13.3°C. In the second replicate, animals fed high quality food aggregated at an average temperature of 16.9°C, while animals fed low quality food aggregated at 13.6°C.

Furthermore, food quality differences caused contrasting daily behavior in *Daphnia* in the presence or absence of fish kairomone (Fig. 4). After the final day, animals fed high quality algae were consistently higher in the water column; they aggregated at an average daytime temperature of 17.6°C. Those fed low quality food were much lower in the water column and aggregated at an average daytime temperature of 14.5°C (all observations included).

Average daytime location was calculated for all eight treatments (Fig 5). Animals fed high quality food in the presence of kairomone aggregated near the surface independently of food location (HT+ and HM+). Animals fed high quality algae at the surface drip without kairomone (HT0) also were high in the water column, but according to a Tukey HSD test, were not significantly different from HT+ and HM+. Animals fed low quality algae in the presence of kairomone remained low in the water column (LT+ and LM+) independently of food location. Animals fed high quality food from the mid-tube drip without kairomone (HM0) were not significantly different from LT+ and LM+.

When scaled according to temperature, *Daphnia* fed high quality food with kairomone (HT+ and HM+) aggregated in warmer water (17.8°C) than those fed low quality algae with kairomone (LT+ and LM+) (15.3°C) (Fig. 6). Animals did not aggregate at significantly different temperatures when kairomone was absent. There was no variation of thermal affinity among treatments without kairomone, mainly because there was much variation in the LM0 treatment.

Interactions existed between several of the main effects. Notably, there were interactions between food quality and presence/absence of kairomone (Fig. 7A), food quality and time (Fig. 7B), and food drip location and kairomone presence or absence (Fig. 7C). When kairomone was present, *Daphnia* daytime spatial location varied depending on food quality. When kairomone was absent, *Daphnia* tended to remain near the thermocline, irrespective of food quality. Migratory behavior was more pronounced at the end of the experiment. Finally, *Daphnia* responded to food drip location when kairomone was present but not when it was absent: animals were found near the thermocline irrespective of food drip location.

Discussion

While it is well-known that many factors influence diel vertical migration, the idea that algal quality may play a role in *Daphnia* habitat selection has not been significantly considered until recently. Because lakes may exhibit strong vertical heterogeneity in algal species (Descy et al. 2000) and chemical composition, food quality could be among the factors involved in habitat selection. Many studies have assumed, without much evidence of support, that the highest quality nutritional layer is found at the surface (see references in MacKay 1996; Gliwicz 2003), and therefore propose that one of the major reasons for the nighttime ascension of zooplankton is to feed "where food levels and food quality are both higher" (Gliwicz 2003 p. 34). However, though the epilimnion is usually warmer than the hypolimnion, it may not always be nutrient-rich from the standpoint of planktonic herbivores. Williamson et al. (1996) were among the first to propose that the highest quality of algae may been found in layers other than the epilimnion. Therefore, there are a variety of habitats that zooplankton may experience during the daytime, including: a nutrient-rich, warm epilimnion; a nutrient-poor, warm epilimnion; a nutrient-rich, cold hypolimnion; and a nutrient-poor, cold hypolimnion. Each of these potential environments yield different trade-offs that zooplankton experience in their diel cycle.

In her 2008 paper, Reichwaldt demonstrated that mature *Daphnia* detect high quality food and change foraging patterns when in a heterogeneous environment. My study reinforced this finding by demonstrating that adult *Daphnia* are able to detect the nutritional quality of their food, and that they are able to vary their spatial location based on food quality. In this experiment, *Daphnia* aggregated in warm surface water if food quality was high and kairomone was present; thus, under these conditions *Daphnia* did not migrate to cooler water in the presence of fish kairomone. Animals given high quality food aggregated in surface waters even if food was delivered mid-tube and kairomone was absent. In contrast, animals given low quality food were migratory, even if kairomone was present. Low quality food yielded aggregations in deeper waters where mean temperature was approximately 14.5°C. In general, the location and thermal environment of *Daphnia* aggregations appeared to depend more on food quality and location than on kairomone. This observation has profound implications for natural populations because both *Daphnia* birth rate and somatic growth rate depend on temperature and food supply (Hall 1964). Food supply plays a large role in setting the birth rate at all temperatures, and indirectly establishes a critical upper limit for mortality by predation as can be illustrated with curves derived from Hall (1964) (Fig. 8). Thus, those populations fed high quality food may have high birth rates because *Daphnia* birth rate depends exponentially on temperatures (Hall 1964); those fed poor quality food should have much lower birth rates due to both the food quality and the cooler average temperature that those *Daphnia* experienced.

Therefore, in this experiment, when kairomone was present, the high food quality, coupled with the warm water temperature, seemed to override the benefits of seeking refuge in the dark hypolimnion during the daytime. Those animals that remained in the epilimnion for the entire 24-hour cycle could be viewed as "epilimnion (epi)-residents."

Growth rates for these animals may be very high; in Sterner et al.'s (in prep) extensive study examining the effects of algal quality, quantity, and temperature on *Daphnia pulex* growth rate, animals grew approximately 0.334 μg/d, a growth rate nearly 1.8 times higher than its migratory counterpart would have, had it spent 12 hours each day in the warm epilimnion (20°C) and 12 hours in the cool hypolimnion (5°C).

However, in the presence of low quality food, remaining in the warmer epilimnion during the daytime reduced animal growth rate. Epi-residents fed low quality food had growth rates that were less than one-half the growth rates of epi-residents fed high quality food (0.334 μg/d vs. 0.159 μg/d). The trade-off faced is: Why risk predation by visually-hunting predators when growth rate will be low anyway? In this case, it was more favorable to migrate: remain in the hypolimnion during the daytime, and migrate into the warmer surface waters at night time. In addition, animals that were fed low quality food and remain in warm water for the entire day have higher metabolic demands than their migratory counterparts. It is possible that the low-quality food present in the epilimnion (coupled with the warm epilimnetic temperatures) is not able to sustain *Daphnia* growth and development. Thus both in terms of metabolism and predation risk, when food quality is low, the hypolimnion seems to be the preferred daytime habitat. These animals could thus be viewed as classical "migrators."

At any particular time of the day, *Daphnia* are faced with multiple environmental factors that affect and may alter the optimal location within a water column for them to reside. These factors include the presence of different types of predators (e.g. fish and/or *Chaoborus*), temperature, dissolved oxygen concentration, and food quantity and quality.

Some of these variables affect *Daphnia* somatic growth, some affect mortality, and some affect both growth and mortality. Some of these environmental factors differ between day and night while others do not. Some of these factors, particularly temperature and predation, have been studied in detail; the role of nutritional factors in influencing DVM, however, is still poorly understood. While models have been designed to attempt to explain zooplankton migration in response to variations in light (e.g. Ringelberg 1999), predation (e.g. Iwasa 1982), and metabolism (e.g. Enright 1977), to name a few, no models to my knowledge have considered migration in response to nutritional factors. Here, I describe a simple model that was created to better understand the multiple trade-offs involved in habitat selection by *Daphnia*. As in our experiment, we consider DVM in response to temperature, predation, and food quality.

The model considers rates of growth (μ, d^{-1}) and mortality (m, d^{-1}) in two water layers (E = epilimnion, H = hypolimnion) as a function of time of day (D = day, N = night) and quality of food (G = good quality food, P = poor quality food). "Food quality" can signify any relevant nutritional dimension, such as the presence or absence of interfering filamentous algae, but the dimension most directly relevant to this chapter is the P content of the food.

The model assumes that food quality affects growth rate but not mortality rate. Further, it assumes that mortality rate differences in the two water layers are food-quality independent. For analytical tractability, the model assumes additive growth rates during day and night, in spite of evidence to the contrary (Sterner and Schwalbach 2001). Including non-additive growth rates would be beyond the present scope, and would require additional information on how growth during the day and night combine, a phenomenon still very poorly understood.

In general, the long-term growth rate can be written as:

$$\frac{\mu_{X,Y} - m_{D,Y}}{2} + \frac{\mu_{X,Y} - m_{N,Y}}{2} \qquad \text{(Eq. 1)}$$

where X refers to the quality of food (G or P) and Y refers to the water layer (E or H) inhabited during that particular time of day (D or N) (see also Table 3). Using this model, different migration strategies can be compared to see which behavioral pattern yields the highest day/night averaged growth rate. In the following, we analyze only conditions where the food quality is homogeneously distributed in the water column, similar to the experimental conditions in this chapter.

First, consider two different strategies "migrating" and "epi-resident behavior" "Migrating" refers to the traditional pattern of inhabiting the hypolimnion in the day and the epilimnion at night; the model could be used to analyze reverse migrations, too, but those are not considered here. Animals should remain epi-resident when their long term growth when inhabiting the epilimnion during day and night exceeds the long term growth when migrating:

$$\frac{\mu_{X,E} - m_{D,E}}{2} + \frac{\mu_{X,E} - m_{N,E}}{2} > \frac{\mu_{X,H} - m_{D,H}}{2} + \frac{\mu_{X,E} - m_{N,E}}{2} \qquad \text{(Eq. 2)}$$

Simplifying (2), remaining a resident in the epilimnion when the water column contains homogeneous food is expected when

$$\mu_{X,E} - m_{D,E} > \mu_{X,H} - m_{D,H} \qquad \text{(Eq. 3)}$$

or upon further rearrangement,

$$\mu_{X,E} - \mu_{X,H} > m_{D,E} - m_{D,H} \qquad \text{(Eq. 4)}$$

The epilimnion-resident strategy is thus favored when the gain in growth from being in the warm epilimnion is greater than the increase in mortality experienced in that layer during the day, a commonsense outcome of the model that relates to the well-known growth/mortality trade-offs involved in DVM (e.g. Huntley and Brooks 1982; Johnson and Jakobsen 1987). Conditions like these may be expected if visually-feeding predators are rare or absent, where temperature differences between the layers are large, or both. Using similar equations, we can also compare the strategy of remaining in the hypolimnion vs. a traditional migration. The condition favoring remaining a hypolimnion-resident compared with migrating is:

$$\mu_{X,E} - \mu_{X,H} < m_{N,E} - m_{N,H} \qquad \text{(Eq. 5)}$$

The hypolimnion-resident strategy is thus favored when the decrease in growth from being in the cold hypolimnion is less than any increase in mortality in the epilimnion vs. the hypolimnion during the night.

Equations 4 and 5 are commonsense statements, consistent with most general overviews of DVM in the literature, which highlight the major trade-off of growth vs. mortality in the warm, epilimnion well-lit in the daytime vs. the cold, always dark hypolimnion. Less obvious, however, these two equations also serve to demonstrate the following: when the absolute difference in growth rates in the two water layers is small, (such as when temperatures are similar) remaining in one or the other layer is less likely to be an optimal solution; both inequalities are harder to satisfy and DVM is favored. Small absolute differences in growth rate may also arise when the water column is homogeneously filled with poor quality food, such that growth rates would be low in both the epilimnion and hypolimnion, in spite of perhaps large temperature gradients. The model thus predicts that whether DVM should be a favorable strategy will be highly dependent on nutritional factors that might limit growth rates. This simple result seems not yet to have been pointed out in the literature.

Turning now to the result of the experiments in this chapter, *Daphnia* fed good food throughout the water column remained in the epilimnion. A version of the model consistent with that observation is:

$$\mu_{G,E} - \mu_{G,H} > m_{D,E} - m_{D,H} \qquad \text{(Eq. 6)}$$

In contrast, *Daphnia* fed poor quality food throughout the water column migrated. A version of the model consistent with that observation is:

$$\mu_{P,E} - \mu_{P,H} < m_{D,E} - m_{D,H} \qquad \text{(Eq. 7a)}$$

and:

$$\mu_{P,H} - \mu_{P,E} < m_{N,H} - m_{N,E} \qquad \text{(Eq. 7b)}$$

Combining equations 6 and 7a means:

$$\mu_{G,E} - \mu_{G,H} > \mu_{P,E} - \mu_{P,H} \qquad \text{(Eq. 8)}$$

Equation 8 specifies a condition for the behavioral patterns observed here: migration in low but not high quality food. For this to be an optimal behavioral pattern, the growth differential between the two layers must be large for a water column containing high quality food, but low for a water column containing low quality food. For any given thermal gradient, such a difference is to be often expected: when food quality is low, growth rates are low, independent of temperatures, so growth differentials are small. In contrast, when food quality is high, growth rates should be more strongly temperature dependent. Temperature- and food-quality-dependent growth rates similar to these have been observed (Sterner et al. in prep).

Note that Equation 8 refers to growth rates but not mortality rates. There is also a mortality-rate condition for migration in poor but not high quality food, which can be obtained by combining Eqs. 7a and 7b:

$$m_{N,E} - m_{N,H} < m_{D,E} - m_{D,H} \qquad \text{(Eq. 9)}$$

Equation 9 states that the difference in mortality rates between the two water layers (food quality independent) is higher in the day than in the night. This condition is consistent with the presence of visually-feeding predators and thus is a different way of expressing that fish predation is a necessary factor to induce a normal migration.

This modeling exercise thus indicates that the behavioral patterns observed in these experiments are likely robust; they are likely to be found in many conditions. It seems that, in general, poor food quality should promote DVM behavior, even when the water column is homogeneous in (temperature-corrected) nutritional state.

It is clear from this study that the quality of food (and in the absence of kairomone, also the food location) regulate the thermal affinity of these animals. *Daphnia* do seem to alter their migratory pattern based on the type of food encountered in their environment. As shown in the model, inhabiting the epilimnion of the water column during the daytime is advantageous only when the nutritional quality of the food in that environment can sustain the animal's growth rate. This also has implications for population dynamics. When birth rate and death rate are equal, barring emigration or immigration, the population size is stable. When mortality due to predation from inhabiting the upper layer during the daytime is larger than the birth rate, the population size will begin to decline. Thus, not only must the growth rate in the epilimnion be high enough to maintain the population size, mortality from predators must be low enough to keep the population size stable (or growing). This critical mortality rate is a major factor in determining the dynamics of the overall population.

Future studies should consider if behavior is consistent throughout organismal ontogeny. This study has confirmed the ability of mature *Daphnia* to alter their diel migrations based on algal quality; but what about juveniles? Many studies have demonstrated that juvenile *Daphnia* do not exhibit a clear migratory pattern (e.g. Wright et al. 1980), though recent studies indicate that juvenile *Daphnia* may indeed change their vertical position in response to stimuli (Winder et al. 2004). The behavior of juvenile *Daphnia* in response to vertical variations in food quality was not known. Do juvenile *Daphnia* lack a behavioral response to varying food quality (as they lack a behavioral response to other treatments), or do the juveniles exhibit a "high-P seeking" behavior as do the mature organisms?

In addition, it is known that various zooplankton have varying internal P contents (ex. Main 1997; Elser et al. 2000; Sterner and Elser 2002). It would be valuable to determine whether or not the detection and position adjustment that was noted in *D. magna* is also present in other species of *Daphnia* that have varying P body contents (such as *D. galeata* with its lower body C:P or *D. pulicaria* with its higher body C:P, Sterner and Elser 2002) as well as other migratory and non-migratory zooplankton (e.g. *Bosmina*, *Chaoborus*, and/or copepods). This study also provides background information for increasing the understanding of homeostasis in zooplankton. How does the migration of animals through nutritionally varied waters affect the internal reserves of, for example, phosphorus? Would a hypo-resident remain more stable in internal P-content than a migratory animal, since the hypo-resident remains in a higher P environment than its migratory counterpart? This experiment lays the groundwork for future studies considering the effects of varying nutritional quality of food and its behavioral and physiological effects on *Daphnia* DVM.

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Table 1. Effects, degrees of freedom, F and p-values from an analysis of variance (ANOVA) of the first experimental repetition.

Effect	DF	F Value	Pr > F
Food quality	1	25.68	0.0010 ***
Location	1	26.37	0.0009 ***
Food * Location	1	0.00	0.9573
Kairomone	1	0.01	0.9452
Food * Kairomone	1	21.86	0.0016 **
Location * Kairomone	1	23.99	0.0012 **
Food * Location * Kairomone	1	0.05	0.8269
Time	11	2.23	0.0200 *
Food * Time	11	5.74	<0.0001 ***
Location * Time	11	0.80	0.6360
Food * Location * Time	11	1.96	0.0434 *
Kairomone * Time	11	1.31	0.2359
Food * Kairomone * Time	11	2.54	0.0082 **
Location * Kairomone * Time	11	3.20	0.0012 **
Food * Location * Kairomone * Time	7	3.65	0.0018 **

Significance at *P < 0.05, **P < 0.01, ***P < 0.001.

Table 2. Effects, degrees of freedom, F and p-values from an analysis of variance (ANOVA) of the second experimental repetition.

Effect	DF	F Value	Pr > F
Food quality	1	44.37	0.0002 ***
Location	1	3.91	0.0835
Food * Location	1	0.99	0.3486
Kairomone	1	2.75	0.1358
Food * Kairomone	1	9.14	0.0165 *
Location * Kairomone	1	6.59	0.0333 *
Food * Location * Kairomone	1	0.20	0.6663
Time	8	2.04	0.0599
Food * Time	8	11.87	<0.0001 ***
Location * Time	8	6.21	<0.0001 ***
Food * Location * Time	8	3.07	0.0068 **
Kairomone * Time	8	4.72	0.0002 ***
Food * Kairomone * Time	8	8.45	<0.0001 ***
Location * Kairomone * Time	8	4.19	0.0007 ***
Food * Location * Kairomone * Time	6	5.63	0.0002 ***

Significance at *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 3. Food quality dependent growth rate and loss (mortality) rate terms for epilimnetic (E) and hypolimnetic (H) *Daphnia* fed good (G) and poor (P) quality food during the day (D) and night (N).

Layer	Growth Rates		Loss Rates	
	Good food	Poor food	Day	Night
Epi	$\mu_{G,E}$	$\mu_{P,E}$	$\mu_{D,E}$	$\mu_{N,E}$
Hypo	$\mu_{G,H}$	$\mu_{P,H}$	$\mu_{D,H}$	$\mu_{N.H}$
	Independent of day/night		Independent of good/poor food	

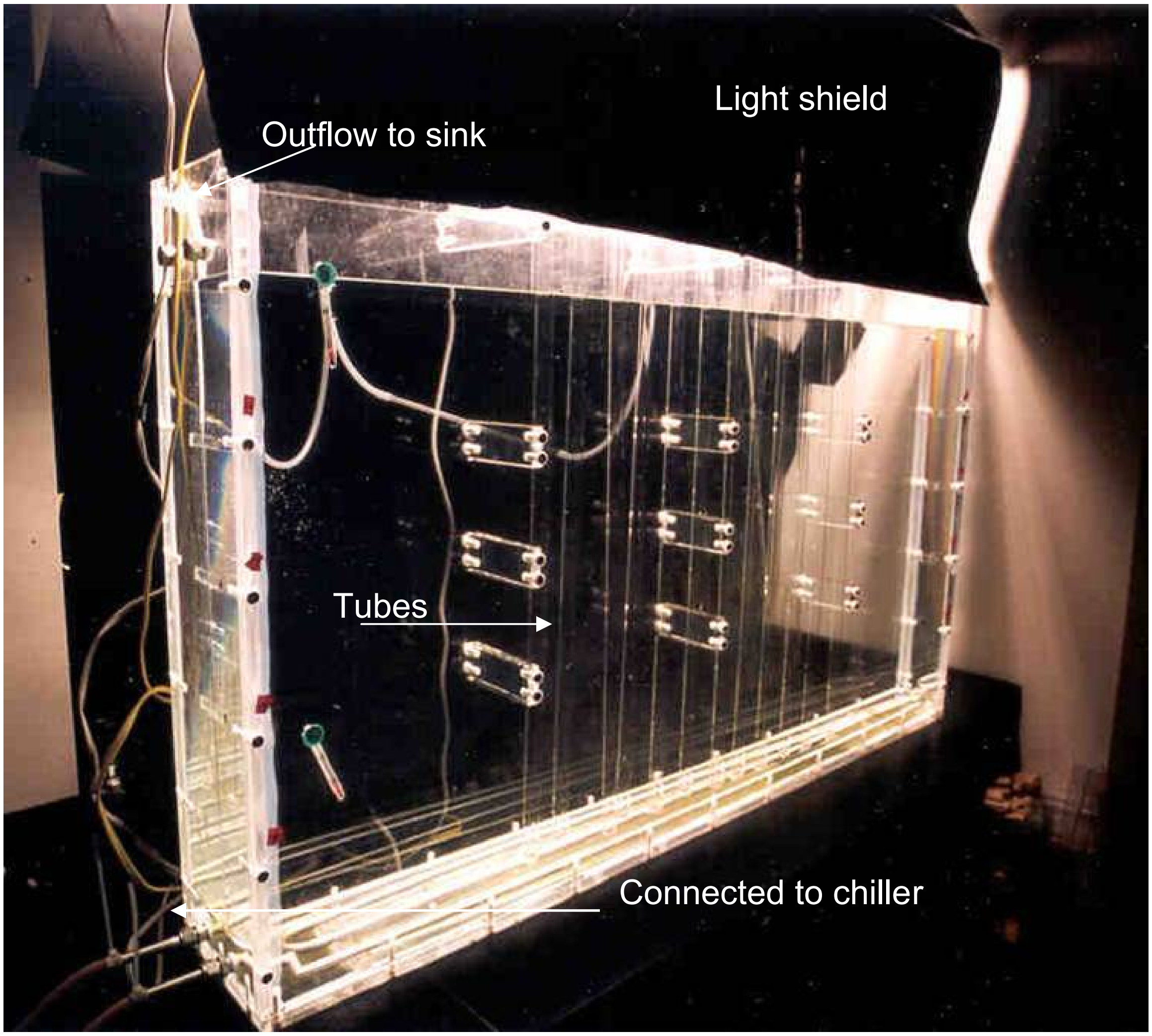

Figure 1a. Photograph of the plankton organ. Photo by R. Anderson.

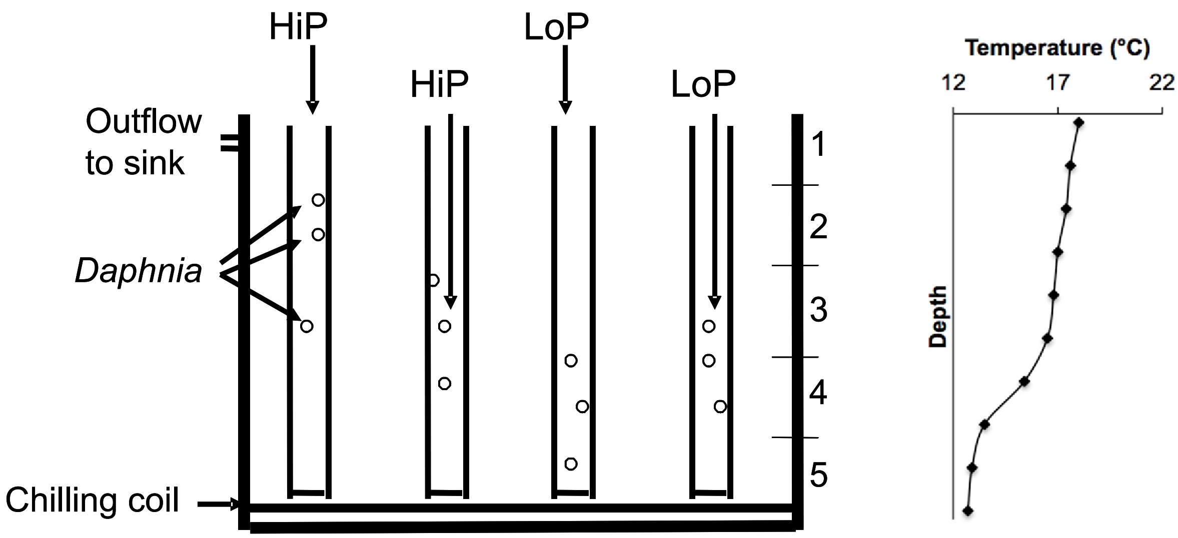

Figure 1b. Schematic of plankton organ and experimental design. HiP represents high quality (high phosphorus) algae (C:P ~ 150) while LoP represents low quality (low phosphorus) algae (C:P ~ 1500). Arrows under treatments indicate drip location- either at the surface or the middle. These four treatments were used both with and without the introduction of fish kairomone. Solid lines and numbers 1-5 indicate boundaries between temperature layers in the plankton organ.

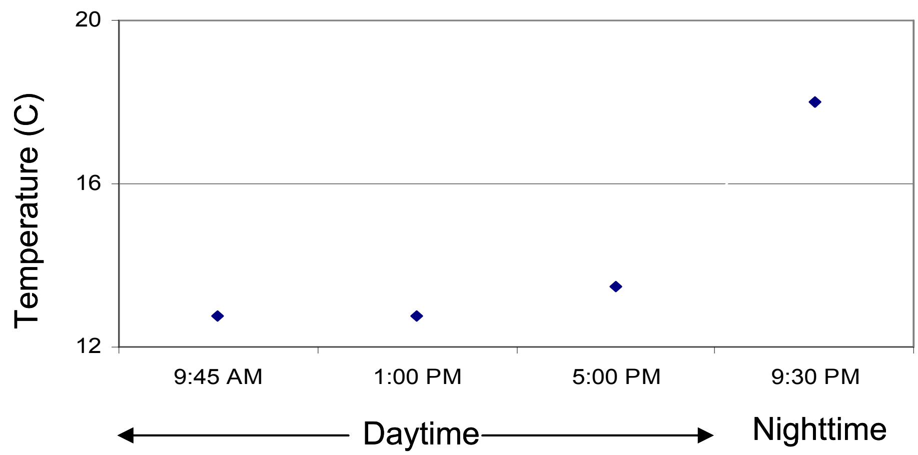

Figure 2. Traditional DVM with *Daphnia* inhabiting lower, (and thus cooler) layers of the water column during the daytime and inhabiting the upper (and warmer) layers of the water column during the nighttime. Data collected May 10, 2002.

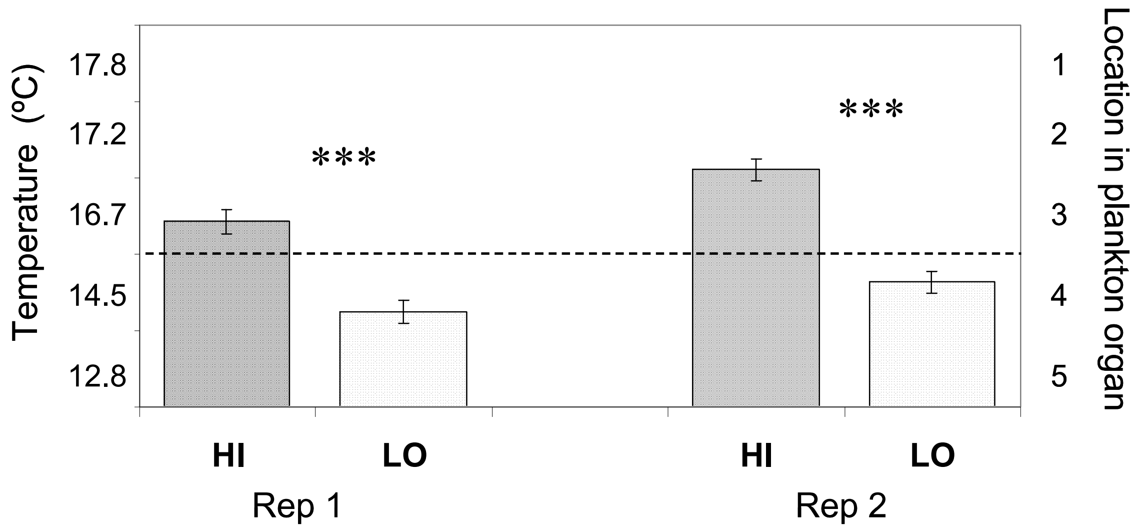

Figure 3. Average daytime spatial location (mean ± SE) of *Daphnia* in response to quality of algae fed during two repetitions. HI represents high quality algae (C:P ~ 150), LO represents low quality algae (C:P ~ 1500), and the dashed line represents the thermocline. Both repetitions were significant at the α = 0.001 level.

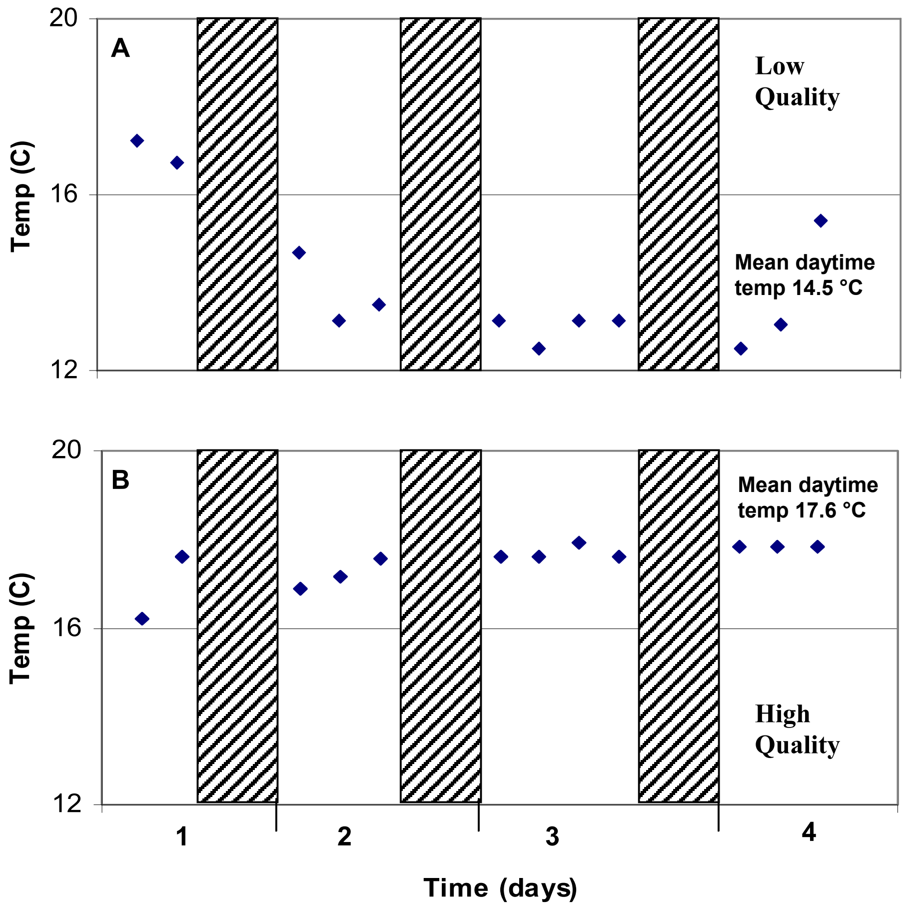

Figure 4. Contrasting chronic behavior due to food quality in the presence of kairomone. *Daphnia* aggregated around 14.5°C when fed low quality food (LM+) and around 17.6 °C when fed high quality food (HM+). Animals from both treatments migrated to the surface (approximately 18.0 °C) at nighttime (Fig. 2).

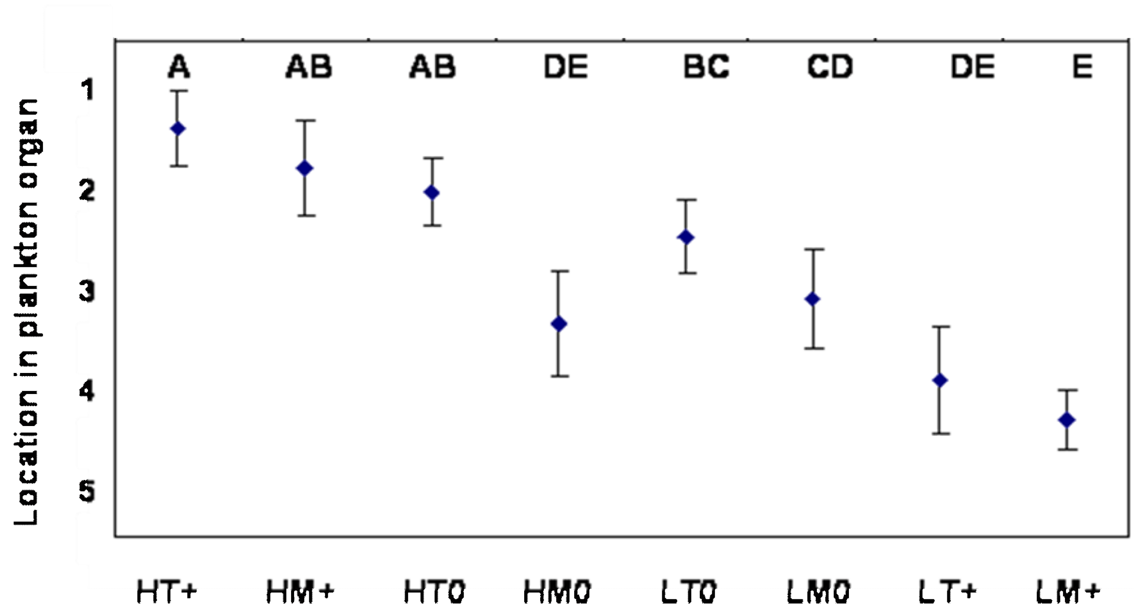

Figure 5. Location of aggregations during daylight for all 8 treatments (mean ± standard error). Treatments are labeled according to quality of food (high (H) or low (L)), location of drip (top (T) or middle (M)) and presence (+) or absence (0) of fish kairomone in the tube.

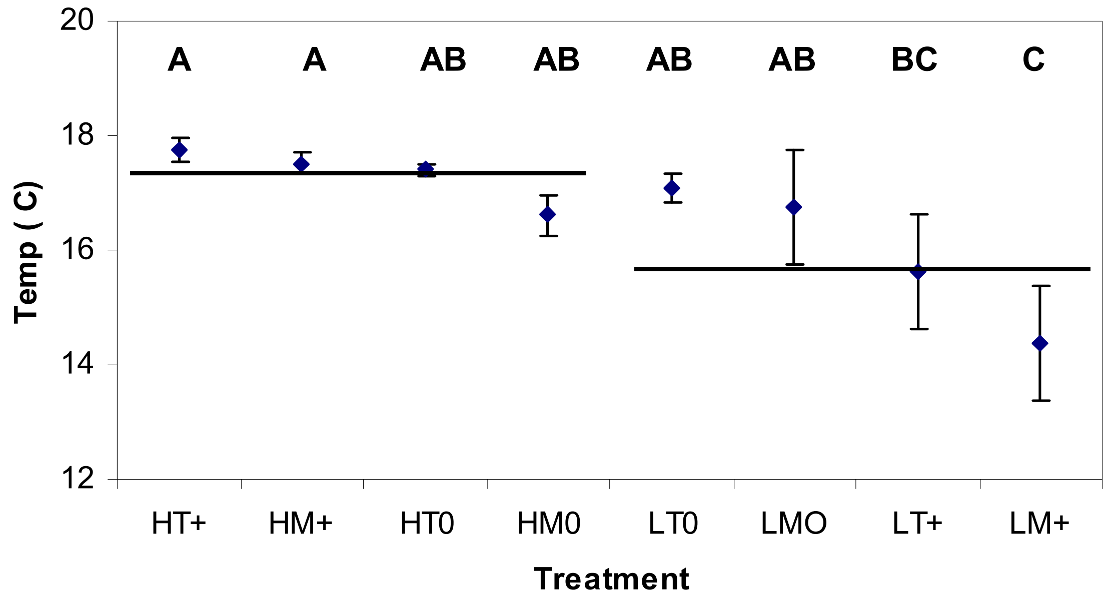

Figure 6. Temperature affinities for all 8 treatments, with mean ± standard error. Treatments labels as in Fig. 5. The mean was 17.2 ± 0.5°C for *Daphnia* fed high quality food and 15.9 ± 1.5°C for those fed low quality food.

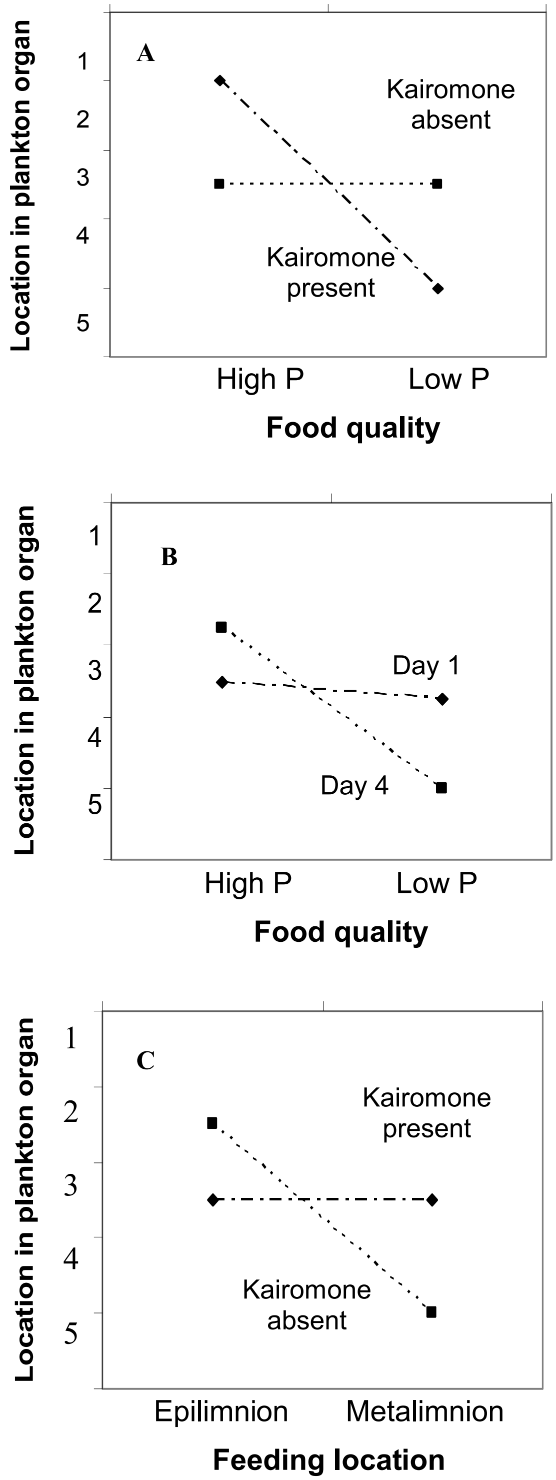

Figure 7. Interaction plots. (A) Interaction between animals fed high and low quality food in the presence or absence of kairomone. In the presence of kairomone, *Daphnia*

fed high quality food aggregated higher in the water column than those fed low quality food. When kairomone was absent, animal location was similar, irrespective of food quality. (B) Interaction between animals fed high and low quality food and time. At the beginning of the experiment, animal daytime location was similar, irrespective of food quality. At the end of the experiment, animals fed high quality food aggregated higher in the water column than animals fed low quality food (Fig. 4). (C) Interaction between the presence or absence of kairomone and feeding location. When kairomone was present, animal daytime location was similar, irrespective of feeding location. When kairomone was absent, *Daphnia* daytime location varied depending on drip location. When food was introduced at the epilimnion, *Daphnia* aggregated higher in the water column; when food was introduced at the metalimnion, *Daphnia* aggregated lower in the water column.

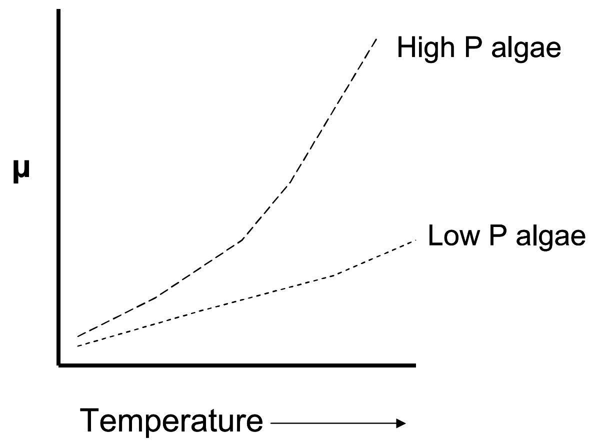

Figure 8. Graphical representation of zooplankton growth rate (μ) and temperature of water when fed high- or low-P food.

Chapter 3

Effects of food quality and temperature on juvenile *Daphnia* behavior and development

Abstract

Variations in temperature and food quality affect growth and development of *Daphnia*. While it is known that the phosphorus content of food, a measure of food quality, affects the thermal affinities and habitat selection of adult *Daphnia* (as discussed in Chapter 2 of this dissertation), the effects of food quality on juvenile *Daphnia* habitat selection is unknown. Experiments described in this chapter were performed to determine if food quality affected the spatial location, growth, fecundity, and net reproductive rate of a population of juvenile *Daphnia* within a temperature-stratified, large-scale plankton organ apparatus.

During daylight, young juvenile *Daphnia* remained in warm surface water, despite variations in food quality. Older juveniles fed P-deficient food moved into colder, deeper water as they aged over a span of six days, though the trend was not significant. The thermal affinities of juveniles fed a P-rich diet did not change over time. Body mass, fecundity, and net reproductive rate were all found to be positively correlated

with increased environmental temperature, and fecundity and net reproductive rate were positively correlated with increased food quality. Variation in net reproductive output among the treatments was highly significant and appeared to be driven by clutch size, rather than by survivorship.

Introduction

Understanding the relationships and dynamics between individuals and their environment is a major focus of ecology. In many lakes, zooplankton exhibit diel vertical migration (DVM) patterns, spending the nighttime in the warmer, well-lit, upper waters of the epilimnion and the daytime in the cooler, poorly-lit, lower waters of the hypolimnion. When migrating through a vertical column of water, zooplankton encounter a range of conditions including variations in light, temperature, and algal quantity and quality. Studies have examined the importance of temperature (McLaren 1963; Calaban and Makarewicz 1982; Makino and Ban 1998), light (Ringelberg and Van Gool 2003; Ringelberg 1999; Winder and Spaak 2002; Hanazato et al. 2002), predation (Dawidowicz et al. 1990), ontogeny (Kessler and Lampert 2004), and food quantity (Pijanowska and Dawidowicz 1987; Gliwicz and Pijanowska 1988) in determining migratory amplitude. Only recently has the effect of varying food quality on zooplankton behavior been examined (Chapter 2 of this dissertation; Schatz and McCauley 2007: Reichwaldt 2008). However, to our knowledge, no studies to date have examined experimentally how *Daphnia* mature when allowed to freely move among a variety of temperatures and food qualities.

It is well known that algal abundance is not distributed homogeneously throughout the water column (Orcutt and Porter 1983; Loose 1993). Algal quality (defined as the atomic ratio of carbon to phosphorus) is also not necessarily homogeneously distributed (Williamson et al. 1996). While previous research assumed the epilimnion to be very food-rich (McLaren 1974; Zaret and Suffern 1976; Enright 1977; Stich and Lampert 1981; Guisande et al. 1991), it has since been argued that epilimnetic algae are often of lower nutritive quality, and that algal quality might be better in deeper waters (Williamson et al. 1996; Sterner et al. 1997). The reasoning behind this is as follows: because light enters a lake at the surface and nutrients generally arise from the sediments, mixing must occur for the hypolimnetic nutrients to reach the epilimnion. During midsummer, due to stratification, sufficient quantities of hypolimnetic nutrients are unable to be mixed into the epilimnion, which is usually depleted in nutrients. When light penetrates to lower layers of the lake, metalimnetic and hypolimnetic seston are able to take up these nutrients and produce phosphorus-rich, high-quality food. In contrast, the epilimnion, with its abundant light but fewer nutrients, produces more carbon-rich, lower quality food. Thus, it is possible that, in a nutritionally patchy environment where visually hunting predators are present, seeking refuge in the cool, dark hypolimnion affords *Daphnia* two benefits; 1) the animals experience reduced predation pressure (due to low light), and 2) they may experience enhanced growth rates due to their feeding on high-quality seston, which is absent in the epilimnion.

Daphnia maturation is affected by its habitat selection, and DVM is, quite simply, vertical habitat selection over time. While the majority of DVM studies have focused on adult *Daphnia*, the few experiments that have examined juvenile behavioral patterns indicate that juveniles generally do not migrate (e.g., Wright et al. 1980), that they exhibit migrations of smaller amplitude than adults (McLaren 1963), or that they are more likely than adults to spend the daytime in the epilimnion (Kessler and Lampert 2004). These previous studies documented light regimes, temperatures, and food quantities offered to the animals, but they did not document the nutritional quality (in terms of C:P) of the *Daphnia* food. It is possible that juveniles, like adults, will vary their daytime vertical position within a water column in response to the food quality presented.

Here in one experiment ("*restriction*") I examine the behavioral responses of juveniles within two different, nutritionally homogeneous (high P or low P), but thermally stratified, environments. In this experiment, I determined whether environmental food quality affected the daytime vertical position of juvenile *Daphnia* and, if so, whether their habitat selection affected growth rates or net reproductive rate. I predicted that juveniles in a homogeneously high-P environment would behave similarly to mature *Daphnia* (as discussed in Chapter 2 of this dissertation) and would remain in the epilimnion for a larger percentage of the daytime than would juveniles placed within a homogeneously low-P environment.

A second experiment *("phosphorus addition")* examined the effects of nutritionally heterogeneous environments on *Daphnia* growth rates. I predicted that *Daphnia* presented with a nutritionally heterogeneous environment (low quality food in the epilimnion and high quality food in the hypolimnion) would have higher growth rates than *Daphnia* that resided in a homogeneously nutrient-poor environment. I predicted that the increased growth rate in the nutritionally heterogeneous treatment would be due to *Daphnia*'s ability to exploit both the high temperatures and the high food quality available in different strata of the water column.

Methods

All experiments were conducted in a temperature-stratified plankton organ apparatus (Fig. 1) that was designed to simulate a stratified lake environment. The basic design of the plankton organ was similar to that described in Dawidowicz and Loose (1992a; 1992b); it was 0.93 meters tall, 0.24 meters wide, and 1.55 meters long. The plankton organ was kept in an isolated room. Plankton organ tubes were suspended in the tank and were made of clear plexiglass; thus, visual observation of *Daphnia* through the tubes was possible. In order to visually determine *Daphnia* position within the plankton organ, the tank was divided into five horizontal sections (top = 1, bottom = 5); these sections were outlined with red string on the outside of the tank so that section markings would not impede zooplankton movement. A temperature gradient was created within the plankton organ in order to simulate a lake's thermocline; the upper portion (epilimnion) remained at room temperature (18.1°C ± 0.4°C), and the lower portion (hypolimnion) was chilled to 12.5°C ± 0.4°C. Prior to *Daphnia* introduction, the tubes were filled with COMBO medium (Kilham et al. 1998) without nitrogen or phosphorus.

Low- and high-quality (in terms of phosphorus) algae were produced by adjusting the N:P ratio of the influent medium and the dilution rate of the laboratory chemostat reaction vessels (Urabe et al. 1997). The protocol to create the algal cultures and details on the composition of the cells can be found in Sterner (1993), Sterner and Smith (1993), and Sterner et al. (1993).

A clone of *Daphnia magna* from the Großer Binnensee, a brackish lake in Northern Germany that contains dense populations of fish (Lampert 1991), was used in the experiments. This *Daphnia* population has been maintained in long-term cultures at the Max Planck Institute for Limnology, Germany, and at the University of Warsaw, Poland (Loose and Dawidowicz 1994). Experimental *Daphnia* were fed a solution consisting of COMBO media mixed with either low-P (LoP, C:P = ~1500) or high-P (HiP, C:P = ~150) *Scenedesmus obliquus*. Carbon contents of the LoP and HiP algae were determined daily; appropriate quantities of algae were added to the media to ensure that the carbon content of each food source was 2 mg C/L. Algae in media were introduced continuously at the top of each of the tubes using a multi-channel peristaltic pump, and the pump was set to fully exchange the media in each plankton organ tube twice daily. The volume within each plankton organ tube remained constant. The introduction of food and media into the top of each plankton organ tube caused the media within the tube to be forced out the bottom tube mesh and into the plankton organ water bath. Thus the algae within each tube both settled naturally and were further pushed out the tube bottom through the introduction of fresh algae and media.

In order to ensure migration, DVM studies frequently introduce a chemical cue indicating the presence of a predator (Loose et. al 1993). A chemical cue in the form of "fish water" was added to each of the plankton organ tubes. The fish water (hereafter referred to as kairomone) was P-free COMBO from an eight-liter carboy that three approximately 7.5cm goldfish had inhabited for at least 12 hours. Fish were fed daily the same clone of *Daphnia* that was used in the experimental tubes because *Daphnia* may emit a chemical stress signal when in the presence of predator cues (P. Dawidowicz, pers. comm.). This chemical stress signal was therefore introduced along with the fish kairomone.

Like the algae, the kairomone was continuously introduced into each of the tubes through the use of the multi-channel peristaltic pump. It was assumed that the kairomone was distributed throughout the tubes; however, there was no way to know the precise distribution of kairomone throughout the tubes, because the composition of chemicals that make up this environmental cue is not known. It should be noted that the algae and kairomone were introduced into each plankton organ tube in separate delivery tubes. This independent introduction ensured that the algae and the kairomone were not mixed before introduction into the plankton organ tube, as prior mixing of the two could potentially alter the nutrient composition of the influent algae.

Restriction experiment

The restriction experiment examined the behavioral responses of juvenile *Daphnia* within two different nutritionally homogeneous but thermally stratified environments. Tubes were randomized and assigned one of six treatments (Fig. 2) (n = 2 tubes per treatment). Each treatment consisted of a specific food quality (HiP = high quality; LoP = low quality) and a physical location within the plankton organ tube (Epi = *Daphnia* restricted to the top half of the tube; Hypo = *Daphnia* restricted to the bottom half of the tube; Mig = able to move freely throughout the entire length of the tube). Specifically, the treatments consisted of: 1) HiP Epi, 2) LoP Epi, 3) HiP Hypo, 4) LoP Hypo, 5) HiP Mig, and 6) LoP Mig. In order to restrict the *Daphnia* to a location within the plankton organ tube, a 35 μm Nitex® mesh barrier was placed approximately 60 cm from the top of the appropriate meter-long tubes. Epilimnetic restriction was achieved by inserting the mesh filter prior to *Daphnia* introduction; hypolimnetic restriction was achieved by transferring animals into the tubes and then inserting the mesh filter into the tube.

Prior to animal introduction, approximately 20 gravid *Daphnia* were placed in a beaker with fresh COMBO medium and high-P algae. Within 24 hours, neonates were transferred into plankton organ tubes. Six *Daphnia* were transferred into each of the epilimnetic-restriction and migratory tubes. Due to the possibility of *Daphnia* mortality during metalimnetic mesh insertion (crushing the animal with the metalimnetic barrier), twelve (rather than six) *Daphnia* were transferred into each of the hypolimnetic-restriction tubes.

The juvenile *Daphnia* were left in the plankton organ tubes for five days. Approximately three times each day, the spatial locations of *Daphnia* in the HiP Mig and LoP Mig treatments were noted. Nighttime locations were not observed due to behavioral response to red light (as discussed in Chapter 2 of this dissertation), and it was assumed that all *Daphnia* remained near the water surface during nighttime. At the end of the five-day experiment, animals were removed from their tubes, checked for ovaries or eggs, dried, and weighed to the nearest tenth of a microgram using a Mettler model LC-P45 microbalance.

The net reproductive output (R_0) was calculated for the first generation of life using the following formula:

$$R_0 = l_5 m_5$$

where l_5 represents the survivorship at day 5 and m_5 represents fecundity at day 5.

All tube and treatment comparisons were based on tube means to avoid pseudoreplication. Comparisons between the daytime spatial locations of the two migratory treatments were made using t-tests. Results were analyzed using factorial analysis. Main effects for the comparisons were food type (high vs. low quality) and location (epilimnion, hypolimnion, and migratory), and the response variables were final animal dry mass, egg output, clutch size, and net reproductive output. Tukey's HSD was used for multiple comparisons of means.

Phosphorus addition experiment

The phosphorus addition experiment sought to examine the effects of vertically varying food quality within a plankton organ tube. Prior to introduction into the plankton organ, approximately 20 gravid *Daphnia* were placed in a beaker with fresh COMBO media and high-P algae. Within 24 hours, 36 *Daphnia* neonates were distributed into the 12 plankton organ tubes (i.e., three per tube; Fig. 3). Tubes were randomized and assigned to one of three treatments (n = 4 replicates per treatment): 1) high-quality food throughout the entire tube (HiP), 2) low-quality food throughout the entire tube (LoP), and 3) low-quality food in the tube's epilimnion and high-quality food in the hypolimnion (LoP + P). All treatments received the addition of kairomone from "fish water" as described in the restriction study, and peristaltic pump rates were set to exchange the contents of each tube once every 24 hours.

Algae for the HiP and LoP treatments were introduced at the top of the tubes using a peristaltic pump (as described in the restriction study). In order to create the LoP + P treatment, low quality *S. obliquus* was introduced via peristaltic pump at the top of the treatment tubes, and 2 ml/l of 80 μM $NaH_2PO_4{\cdot}H_2O$ was continuously introduced just below the thermocline. An experiment performed under the same light and temperature conditions demonstrated that low-quality (P-poor; C:P = 662) *S. obliquus* took up enough bioavailable P (from a 2 ml/l spike of 80 μM $NaH_2PO_4{\cdot}H_2O$) to be converted to high-quality (P-rich; C:P = 316) algae within 30 minutes (Fig. 4). Furthermore, within two hours, enough of the P spike was incorporated into the algae to drop the *S. obliquus* C:P to 164. Thus, in the presence of bioavailable P, within a short timeframe, low-quality *S. obliquus* were converted from high-C, low-P algae into a P-rich, high-quality food. Furthermore, because *S. obliquus* are not motile, they are only able to descend in the plankton organ tube; they are unable to move up in the plankton organ tube. Thus, hypolimnetic algae in the LoP + P treatment should be high-quality, while algae in the epilimnion should remain low-quality.

Daphnia remained in the plankton organ tubes for four days, after which time they were removed from the tubes, dried, and weighed to the nearest tenth of a microgram using a Mettler model LC-P45 microbalance. Data were analyzed using a one-way analysis of variance with three levels (HiP, LoP, HiP + P), and Tukey's HSD was used for multiple comparisons of means.

Results

Restriction study

Daytime locations of juvenile *Daphnia* were determined for the subset of tubes in which animals were allowed to freely migrate between warm and cool layers (migratory tubes). Adult *Daphnia* of the same clone in similar temperature and food conditions as those in this migratory restriction experiment exhibited a strong food quality-dependent propensity to migrate (as discussed in Chapter 2 of this dissertation): adults fed low-quality food exhibited a "typical" migratory pattern (i.e., remaining in the hypolimnion during the daytime and rising to the epilimnion during the nighttime), and adults fed high-quality food remained near the epilimnion at all times.

During the first five days of this experiment, juvenile behavior did not mimic the above-mentioned adult behavior; the spatial locations were not statistically significantly different between the high-P and low-P migratory treatments (Table 1 and Fig. 5). As the animals fed low-quality food matured from day one to day five, the general trend was to descend in the water column during the daytime, thus experiencing cooler average daytime temperatures. Animals were occasionally noted below the thermocline (e.g., observation three on day two and observations two and three on day four); however, the spatial separation between animals in the high-P and low-P treatments appeared to be small. On the final day of the experiment (day 6), *Daphnia* in the low-P treatment were noted well below the thermocline while *Daphnia* in the high-P treatment were noted above the thermocline. However, this difference was not significant.

The final dry mass of juvenile *Daphnia* did not vary significantly among treatments (F (5,6) = 2.85, p = 0.11, Fig. 6). However, final animal dry mass varied significantly with restriction location (two-way ANOVA, F (2,6) = 6.12, p = 0.03). When animal restriction location (irrespective of food quality) was compared using Tukey's HSD, *Daphnia* in the epilimnetic treatments were significantly larger in mass than animals in the hypolimnetic treatments (0.142 ± 0.020 mg vs. 0.060 ± 0.008 mg, respectively). While the restriction location was found to be significant, neither food quality nor the restriction location by food quality interaction were significantly different (F (1,6) = 1.16, p = 0.32 and F (2,6) = 1.01, p = 0.42, respectively). The lack of significant difference between the final mass of animals from the high-P and low-P epilimnetic restriction treatments was surprising, because previous studies have indicated that *Daphnia* are P-limited (Urabe et al. 1997). Small sample size and fairly large variation can not be ruled out as possible reasons for the lack of significance.

The percentage of egg-bearing individuals varied significantly among treatments (F (5,6) = 7.45, p = 0.015, Fig. 7). A multiple comparison of means showed that the HiP Epi treatment was significantly different from the HiP Hypo and the LoP Hypo treatments. No other treatments were significantly different from one another. Regardless of food quality, the percentage of egg-bearing individuals varied significantly with restriction location, with the majority of the animals in the epilimnetic treatments producing eggs and none of the animals in the hypolimnetic treatments producing eggs (two-way ANOVA, F (2,6) = 15.50, p = 0.0043). The effect of varying food quality was nearly significant (F (1,6) = 3.63, p = 0.105), and the location by food quality interaction was not significant (F (2,6) = 1.31, p = 0.33). While egg production appeared to be very different between the groups, the lack of statistical significance was likely due to the high variation among the percent of egg-bearing individuals in the migratory treatments (80% in one tube and 16.6% in the other tube for the HiP Mig treatments and 40% and 16.6% for the LoP Mig treatments).

Daphnia in the epilimnetic and migratory treatments produced an average of 7.3 ± 1.5 and 3.2 ± 0.5 eggs per *Daphnia* in each treatment, respectively, whereas *Daphnia* in the hypolimnetic treatments did not produce eggs. The clutch size varied significantly among treatments (two-way ANOVA, F (5,6) = 30.37, p = 0.0003, Fig. 8). All effects tests were found to be significant: restriction location (two-way ANOVA, F (2,6) = 59.77, p = 0.0001), food quality (two-way ANOVA, F (1,6) = 18.15, p = 0.0053), and location by food quality interaction (two-way ANOVA, F (2,6) = 12.93, p = 0.02). A multiple comparison of means of the six treatments found three groupings. The HiP Epi treatment was significantly different from all other treatments (group A). The two Hypo treatments formed another grouping (group C). The LoP Epi and HiP Mig treatments formed a third group (group B), and the LoP Mig treatment was grouped in both groups B and C.

Net reproductive output varied significantly among treatments (F (5,6) = 34.95, p = 0.00002) (Figure 9 and Table 2) and appeared to be driven by clutch size rather than by survivorship. The proportion of animals surviving to day 5 in the high-P epilimnetic treatment was lowest among all treatments; however, the clutch size was the highest. Thus, the high-P epilimnetic treatment yielded the highest net reproductive output of 7.3 ± 0.0. Survivorship in the low-P migratory treatment was higher than survivorship in the high-P epilimnetic treatment; however, egg production was much lower in the low-P treatment; thus, the net reproductive output was very low among animals in the low-P migratory treatment (2.1 ± 0.15). Survivorship was high among *Daphnia* in both hypolimnetic treatments, however, the net reproductive output of was 0 due to the failure to produce eggs. When the data were compared using Tukey's HSD, the groupings were the same as those identified for the clutch size comparison. The HiP Epi treatment was significantly different from all other treatments (group A). The two Hypo treatments formed another grouping (group C). The LoP Epi and HiP Mig treatments formed a third group (group B), and the LoP Mig treatment was grouped in both groups B and C.

Phosphorus addition experiment

Daphnia mass varied significantly among the three treatments (high-P, low-P, and low-P + P) (F (2,8) = 5.35, p = 0.033, Fig. 10). *Daphnia* in the high-P treatment were nearly twice the mass of *Daphnia* in either the low-P or low-P + P treatments, and multiple comparisons of means using Tukey's HSD confirmed the significant difference between the high-P and the two low-P treatments. Thus, though algal food quality (high-P vs. low-P) affected the growth rate of the animals, the addition of a metalimnetic phosphorus supplement to the low-P treatment did not appear to have an effect during this experiment. This lack of response may indicate either of the following: 1) that despite access to high-P food in the hypolimnion, animals in the low-P + P treatment did not exploit the high-P algae in the tube's hypolimnion, and therefore did not receive any detectable benefit from the metalimnetic P addition or 2) that the benefit received from grazing higher quality algae in the low-P + P environment was offset by the cooler hypolimnetic temperatures.

Discussion

Behavior

Daytime vertical position of zooplankton has been shown to vary with ontogeny (Kessler and Lampert 2004; Voss and Mumm 1999, Reichwaldt 2008). When an animal is young, it is small, and it is therefore less visible to visually-hunting predators than individuals that are larger, that have food in their digestive tract (thus making them more conspicuous [Tsuda et al. 1998]), that are gravid, or that are large and highly pigmented (Zaret 1980). These highly conspicuous animals tend to exhibit a more pronounced vertical migration than do young individuals (Wright et al. 1980). Because adult *Daphnia* modify their daytime vertical position based on food quality in the water column (as discussed in Chapter 2 of this dissertation), it was thought that juvenile *Daphnia* may do so as well. However, in these experiments, perhaps because they are less conspicuous, juveniles did not alter their behavior when in the presence of varying food qualities. These findings are consistent with Reichwaldt's (2008) findings that habitat selection of adult *Daphnia* is affected by food quality whereas food quality does not appear to affect juvenile habitat selection.

Regardless of food quality, when presented with a thermally stratified tube, juvenile *Daphnia* favored the epilimnion, spending more time in the warmer water than in the cooler hypolimnion. On the final day of the experiment, however, it appeared that *Daphnia* were beginning to follow the same migratory pattern that adults have exhibited. As the animals matured, *Daphnia* fed high-quality food remained in the warmer epilimnetic water, whereas *Daphnia* fed low-quality food remained in the cooler hypolimnetic water.

Daphnia may exhibit one of two strategies when comparing the cost associated with increased predation risk and the benefit associated with early reproduction. Zooplankton may face increased risk of predation in the light-filled epilimnetic waters during the daytime *(cost)* in order to more rapidly mature and produce offspring *(benefit)* (e.g., Lock and McLaren 1970; Kirby 1971; Halbach 1973; Keen 1979), or they may minimize predation risk by remaining in the dark hypolimnion *(benefit)* (e.g., Ohman 1990; Bollens and Frost 1991; Bollens and Stearns 1992) but mature at a much slower rate and have lower fecundity *(cost)* (e.g., Orcutt and Porter 1983; Dawidowicz and Loose 1992b).

What effects does varying food quality have on the above cost/benefit analysis? Animals that are fed high-quality food have the ability to maximize their growth rate when in the epilimnion; high-quality food in a warm environment leads to a rapid growth rate and a decrease in generation time. These animals still risk predation by spending the daytime in the well-lit epilimnion, but the increase in growth rate may outweigh the risk of predation. Animals that are fed low-quality food yet reside in the epilimnion are constrained by the nutrients in their food; they are unable to grow at their maximal rate in this warm temperature.

Therefore, in a low-quality food environment, the risk of predation is greater than the benefit of being in warm water. When food quality is low, spending at least a portion of the daytime in the hypolimnion is more beneficial, because the animal is not required to maintain such a high metabolic rate. In addition, some studies have demonstrated that variations in food quality (Sterner et al. in preparation) and quantity (Hall 1964) have a significantly smaller effect on animal growth rate in cool water than at warmer temperatures. Thus, there is a complex interplay between temperature and food quality when predicting *Daphnia* behavior and ideal daytime habitat selection.

Habitat selection varies throughout a daphnid's lifetime. In the presence of predator cues, adult *Daphnia* vary their daytime spatial location in response to varying food quality (as discussed in Chapter 2 of this dissertation). In contrast, this study demonstrates that, under similar conditions, juvenile *Daphnia* do not vary their daytime spatial location in response to food quality. There must be a critical age when *Daphnia* are able to detect the food quality in their environment and then modify their behavioral patterns (through habitat selection). During this critical period, *Daphnia* must "learn" to remain in the warm epilimnion during the daytime only if the food is of sufficient quality and quantity to maintain a rapid growth rate (i.e., when the benefit of rapid growth outweighs the cost of predation). Additional studies into the effects of ontogeny on DVM would greatly benefit the understanding of this phenomenon and could perhaps uncover a migratory cue.

Growth

Juvenile *Daphnia* habitat selection affects mortality rate, growth rate, and fecundity. Selection of a warm, nutrient- and food-rich environment allows for rapid growth, thus increasing growth rate and fecundity. But, as discussed above, what benefit would an animal receive by remaining in the epilimnion if food quality were poor? I predicted that, in the restriction study, animals in the high-quality food/epilimnetic group would have the greatest mass at the conclusion of the experiment. While animals from this treatment were larger than animals from other treatments, the mass difference was not statistically significant. It should be noted that the number of *Daphnia* in the hypolimnetic tubes was double the number of animals in the epilimnetic tubes. However, if food were limited in the hypolimnetic tubes, the dry mass of those animals should have been even lower than it was due to a starvation-induced reduced growth rate. Therefore, I do not believe that food limitation was a factor in the low growth rate of the animals in the hypolimnetic treatments.

In the phosphorus addition experiment I observed (as predicted) a significant difference in animal dry mass between animals fed high-quality food (one treatment) and those fed low-quality food in the epilimnion (two treatments; Fig. 10). Thus, in those experiments, food quality affected animal growth rate. I also predicted a significant mass difference between *Daphnia* in the homogeneously low-quality food treatment and those in the hypolimnetic P-enriched treatment. However, I did not detect a significant difference in final animal mass between these two treatments. Recently, Schatz and McCauley (2007) demonstrated that *Daphnia* have the ability to forage to find high-quality food. Furthermore, Sterner and Schwalbach (2001) found that when *Daphnia* inhabit a vertically patchy environment, the benefits of their P-enriched diet are greater than the additive effect that would be expected (due to "luxury consumption"). In this study, juveniles may not have spent enough time in the hypolimnion to take up sufficient quantities of P (through "luxury consumption") to increase their growth rate. Other possible explanations for the low final mass in the low-P + P treatment include reduced feeding in the P-rich hypolimnion and retarded growth rates due to the cool hypolimnetic temperatures (with the cool temperature outweighing the benefit of the P-rich food). Additionally, while I assumed that the plankton organ remained stratified during the metalimnetic P-addition, the possibility exists that the stratification may have been disrupted, allowing for the diffusion of some of the P spike into the epilimnetic waters.

However, had some of the P spike diffused into the epilimnion, the LoP + P treatment should have been more similar to the HiP treatment, not the LoP treatment. Thus, I do not believe that the possibility of P diffusion explains the lack of significant difference between animals in the LoP and the LoP + P treatment. Future experiments should attempt to quantify the temperature and C:P within all tubes as well as track the behavior of the animals while in the plankton organ tubes (as was done for the restriction study) to ascertain the amount of time *Daphnia* spend in the hypolimnion of the low P + P tubes.

Reproductive output

How might juvenile habitat selection affect net reproductive output? As animal growth rate increases, the time to maturation and subsequently the generation time both decrease. In the restriction study, *Daphnia* that inhabited warm water (either in the epilimnetic-only or the migratory treatments) produced eggs. More interesting, however, was the lack of significant difference in percent of animals producing eggs between the epilimnetic-restriction and migratory treatments. It is not surprising that there was no significant difference in egg production between the epilimnetic and the migratory tubes *within* a food quality, because animals tended to spend the majority of their time in the warmer epilimnion despite the ability of some to migrate the entire length of the tube. I was, however, surprised that the egg-bearing females fed low-quality food produced the same number of eggs on average as *Daphnia* fed high-quality food. It is possible that the *Daphnia* in the study were, in the beginning, living off of their P reserves, as all gravid mothers that provided neonates for the study were reared in high-quality food. Studies in other fields have identified similar transfer of parental nutritional benefits to offspring (e.g., Fox et al. 1995). Perhaps those animals fed low-quality food would have taken longer to produce a second clutch of eggs than those animals fed high quality food. Another possible explanation is that the *Daphnia* in the high-quality food treatments had a more rapid production of eggs than those in the low-quality food treatment, such that, even though the reproductive outputs of the two groups were similar, the generation times were not. In order to better understand the potential differences in net reproductive output among the treatments, future studies should attempt to address the potential confounding factor of the parental history of the juveniles used in the experiments as well as consider running experiments for a longer timeframe.

The differences that were observed in *Daphnia* net reproductive output demonstrated that, in this experiment, net reproductive output was being driven by clutch size and not by survivorship. Survivorship was lowest among those animals in the high-quality, epilimnetic-restriction treatment. However, this treatment yielded the highest net reproductive output due to the large clutch sizes produced. In contrast, *Daphnia* that solely inhabited cool waters exhibited higher survivorship rates, but they produced almost no eggs. While these animals likely would have produced eggs if allowed to remain in the cooler temperatures for a longer period of time, the consequences of life in the cool habitat are obvious. Despite the ability to seek refuge from visually-hunting predators, population growth in cold temperatures is much slower. This slow and low egg production represents the cost of remaining in cooler waters at all times: low reproductive output.

Theoretically, in the laboratory, the epilimnetic population of *Daphnia* fed high-quality food would increase much more rapidly than any of the other populations I examined. But would this happen in the real world? Survivorship in the plankton organ is not based on real predation; only the predator cues (in the form of kairomone) are present. In the real world, would the risk of remaining in the epilimnion during the daytime (predation) outweigh the benefit of increased growth rate and decreased generation time? If survivorship were reduced to 33% and the number of eggs produced per surviving individual remained unchanged (at 9.9 eggs/individual, which is the average for the HiP Epi treatment), net reproductive output would fall to 3.3, which is approximately the same as the net reproductive output of the migratory treatments. Thus, in that case, while there would be an individual benefit to migration, there would be no detectable benefit to migration on a population basis. Future studies that incorporate real predators (instead of predator cues in the form of kairomone) would add greatly to our understanding of daytime spatial location and net reproductive output.

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Table 1. Mean, standard error, and t-tests of mean vertical location of HiP-fed migratory *Daphnia* vs. LoP-fed migratory *Daphnia* in the restriction study. Column values represent spatial location of animals in the plankton organ tubes, with 1 being the top of the tube and 5 being the bottom of the tube. N = 2 tubes/treatment.

	HiP Mean	LoP Mean	HiP SE	LoP SE	t stat	P (T<=t)
Day 1, Time 1	2.50	1.23	4.50	0.01	0.89	0.54
Day 2, Time 1	2.75	1.90	1.13	0.18	1.89	0.31
Day 2, Time 2	1.84	1.80	1.39	1.28	1.00	0.50
Day 2, Time 3	3.24	3.75	0.64	0.02	-0.79	0.58
Day 3, Time 1	3.10	1.97	1.62	0.27	0.90	0.53
Day 3, Time 2	3.50	2.00	3.56	0.05	1.00	0.50
Day 3, Time 3	3.00	2.00	3.92	0.00	0.71	0.61
Day 4, Time 1	3.03	2.35	0.79	0.04	0.87	0.54
Day 4, Time 2	3.67	2.73	0.88	0.55	0.79	0.57
Day 4, Time 3	3.08	3.65	1.69	1.45	-0.32	0.80
Day 5, Time 1	4.17	3.42	1.39	0.01	0.82	0.56
Day 5, Time 2	3.17	3.04	6.73	5.35	0.04	0.98
Day 5, Time 3	3.17	3.00	3.56	2.88	0.07	0.96
Day 6, Time 1	2.25	4.08	1.13	0.67	-1.38	0.40

Table 2. Life table parameters for each tube and each treatment from the restriction study. Treatment food types were low phosphorus (LoP) and high phosphorus (HiP). Treatment locations included being restricted to the tube's hypolimnion (Hypo), being restricted to the tube's epilimnion (Epi), and being able to move freely throughout the length of the tube (Mig). Tubes were sorted in decending order according to reproductive rate.

Treatment	# of *Daphnia*/ tube at experiment start n	Number Surviving To Day 5 ax	Proportion surviving to day 5 lx	# of eggs per surviving individual mx	Net reproductive rate R0 = (lx*mx) R0
HiP Epi	6	4	0.667	11.0	7.3
HiP Epi	6	5	0.833	8.8	7.3
LoP Epi	6	6	1.000	5.5	5.5
HiP Mig	6	5	0.833	5.4	4.5
LoP Epi	6	5	0.833	4.2	3.5
HiP Mig	6	6	1.000	3.3	3.3
LoP Mig	6	6	1.000	2.3	2.3
LoP Mig	6	5	0.833	2.4	2.0
LoP Hypo	12	11	0.917	0.0	0.0
LoP Hypo	12	10	0.833	0.0	0.0
HiP Hypo	12	12	1.000	0.0	0.0
HiP Hypo	12	11	0.917	0.0	0.0

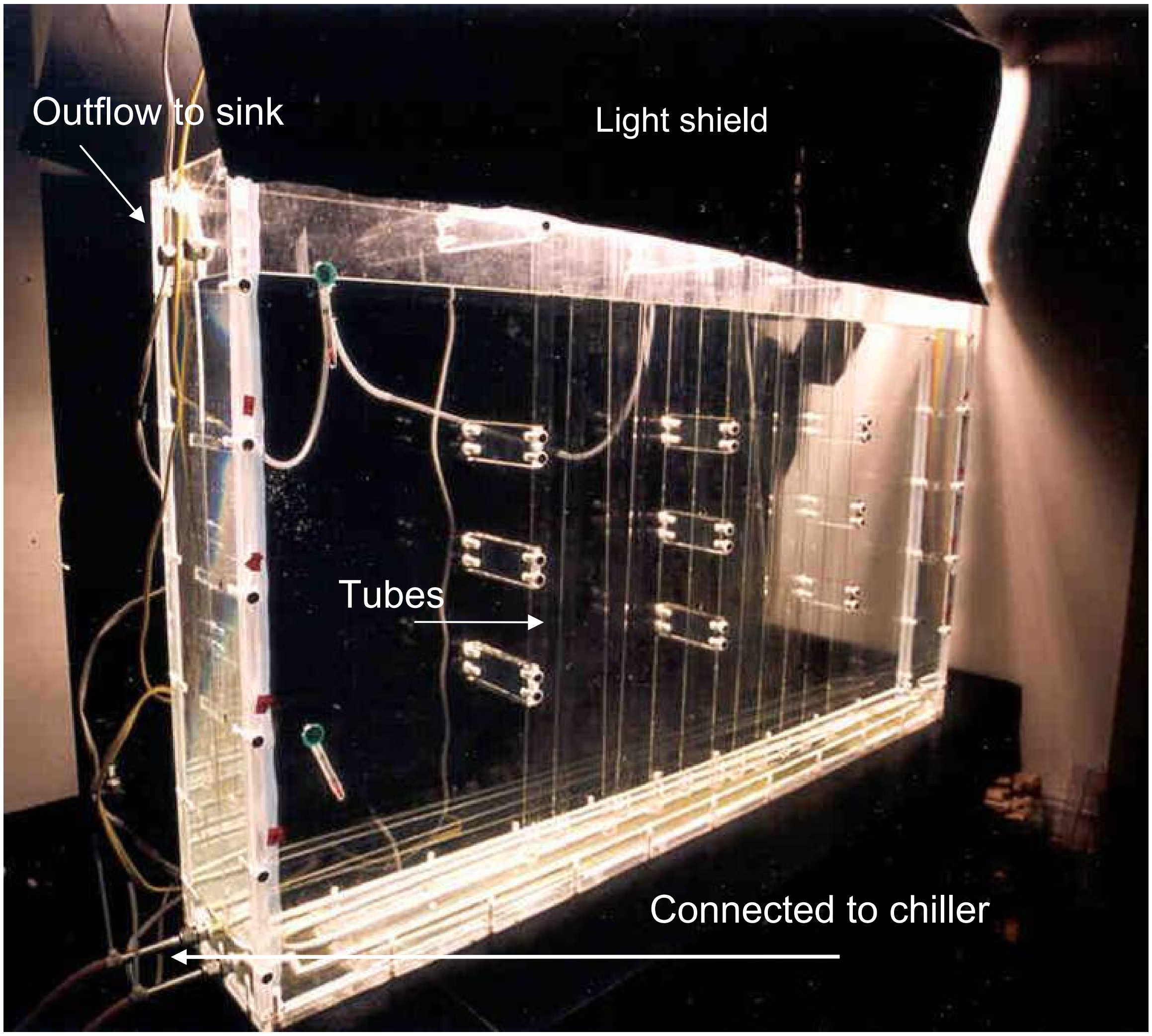

Figure 1. Photograph of the plankton organ. Note the chilling coil on the bottom of the plankton organ, the tubing circulating the hypolimnetic water, and the shield on the light bank. Photo by R. Anderson.

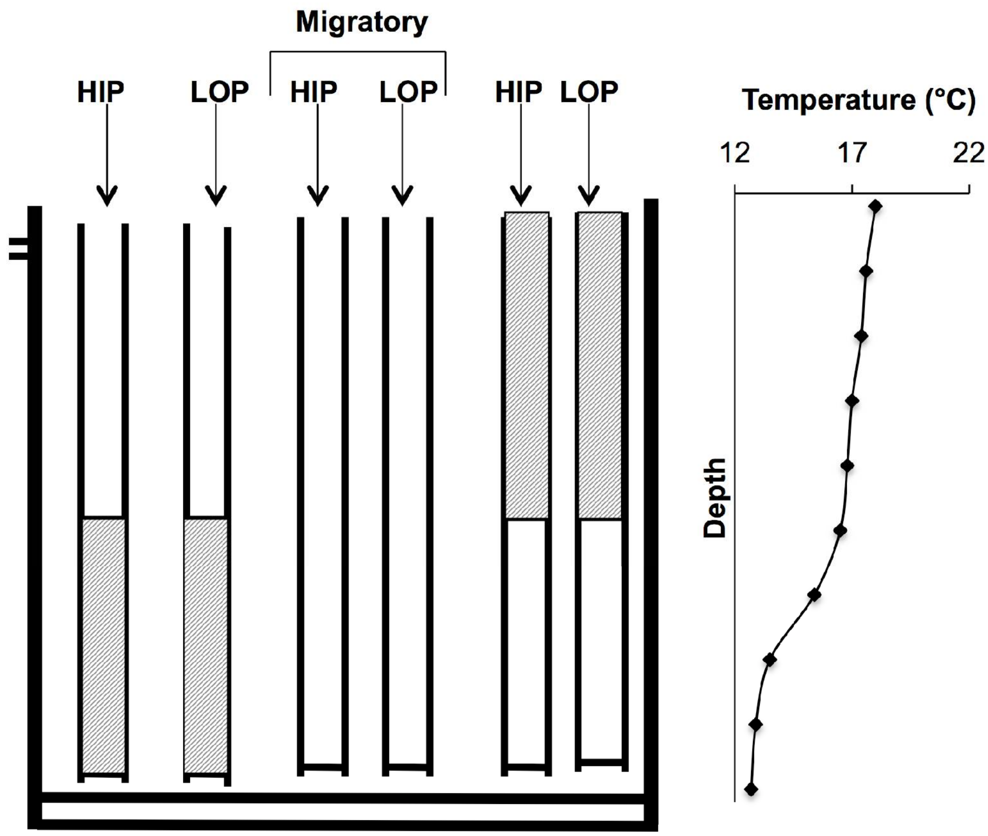

Figure 2. Schematic of plankton organ and experimental design of the restriction study. Two tubes were tested for each treatment and the shaded portions represent areas that were inaccessible to animals. At the beginning of the experiment, six animals were transferred into the treatment tubes that restricted movement to within the epilimnion. Six animals were also transferred into each of the migratory treatment tubes and were allowed to freely move throughout the full length of the tube. Twelve animals were transferred into the treatment tubes that restricted movement out of the hypolimnion. High (HiP) and low (LoP) phosphorus algae were continuously dripped into each of the tubes, and a temperature gradient was maintained throughout the plankton organ. The corresponding temperature gradient is plotted on the right.

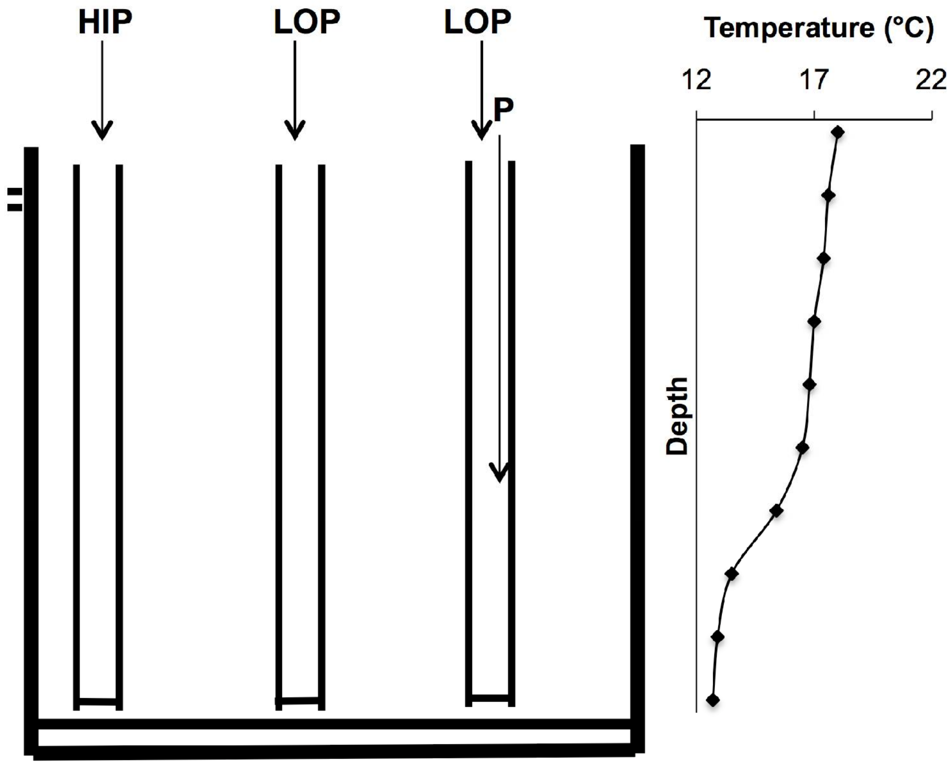

Figure 3. Schematic of plankton organ and experimental design of the phosphorus addition study. HiP represents high-phosphorus algae and LoP represents low-phosphorus algae dripped into the top of the tube. The P on the third tube represents addition of 2 ml/l of 80 μM $NaH_2PO_4 \cdot H_2O$ continuously introduced at the thermocline. Three animals were placed in each treatment tube at the beginning of the experiment, and there were four tubes of each treatment. The temperature gradient is plotted on the right.

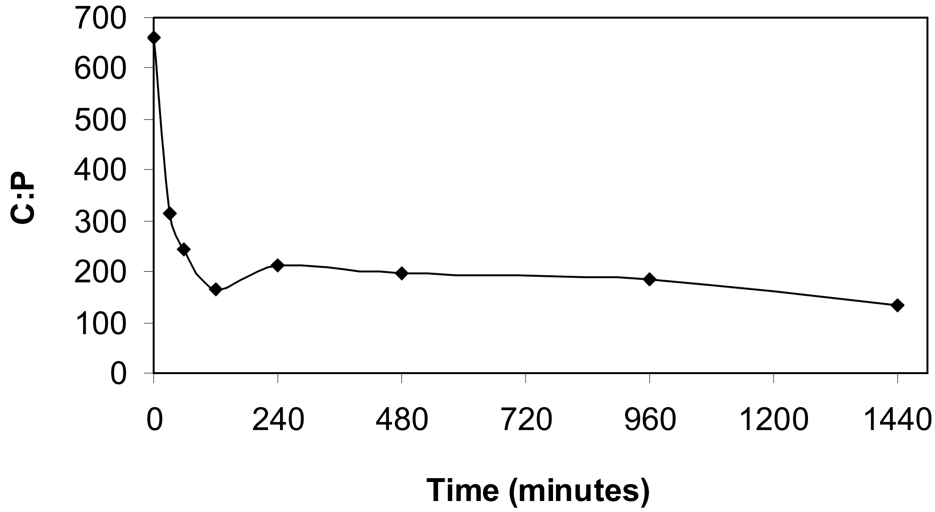

Figure 4. C:P of phosphorus-deficient *Scenedesmus obliquus* after spiking with 2 ml/l of 80 μM $NaH_2PO_4 \cdot H_2O$ at time = 0.

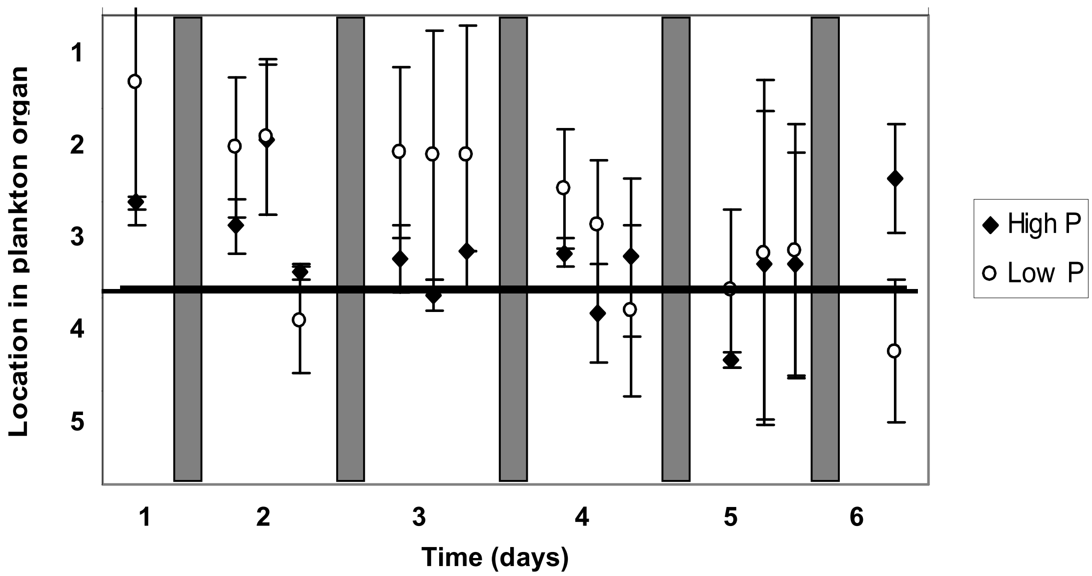

Figure 5. Daytime spatial location (1 = top, 5 = bottom) of juvenile *Daphnia* fed high- and low-quality food and able to migrate through a thermally stratified column of water (treatments "Mig" in the restriction study ± SE, n = 2 tubes per treatment). The thermocline is denoted by the solid black line between sections 3 and 4. No significant location difference was found among any of the comparisons.

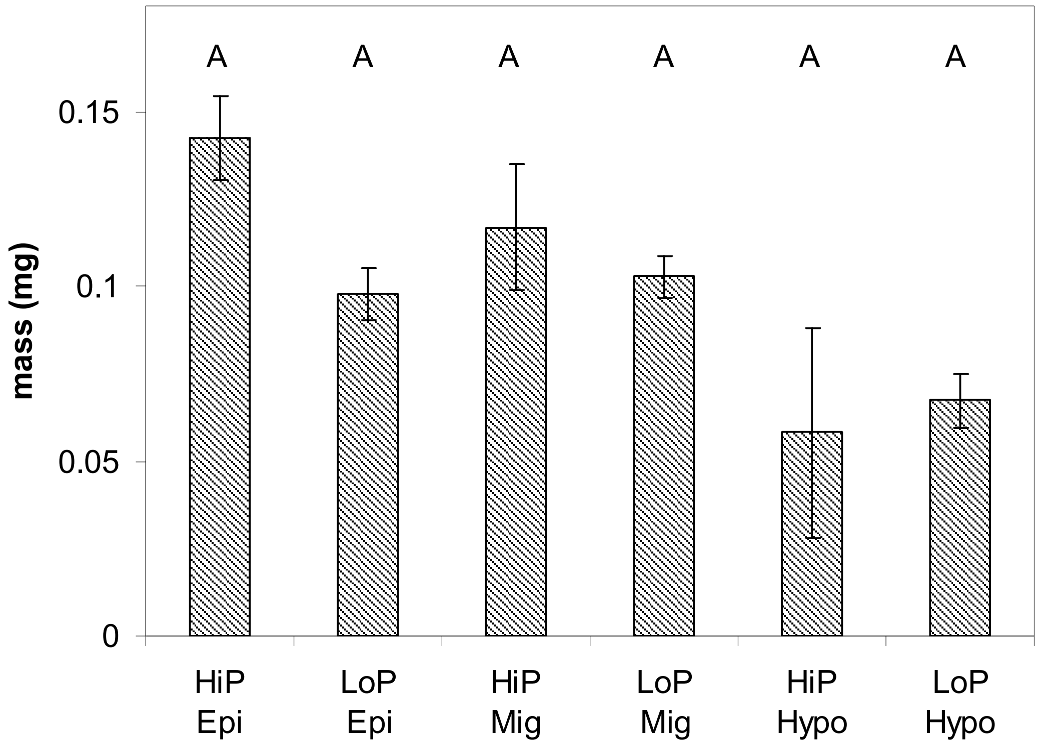

Figure 6. Final mass of *Daphnia* in restriction study (mean ± SE, n = 2 tubes per treatment). High- quality food is represented by HiP and low-quality food by LoP. *Daphnia* restricted to the top of the tubes are labeled Epi, those restricted to the bottom are marked Hypo, and those that had the ability to move throughout the length of the tube are marked Mig. Means were not significantly different (Tukey's HSD, $p > 0.05$) from one another (as noted by common letters above bars.)

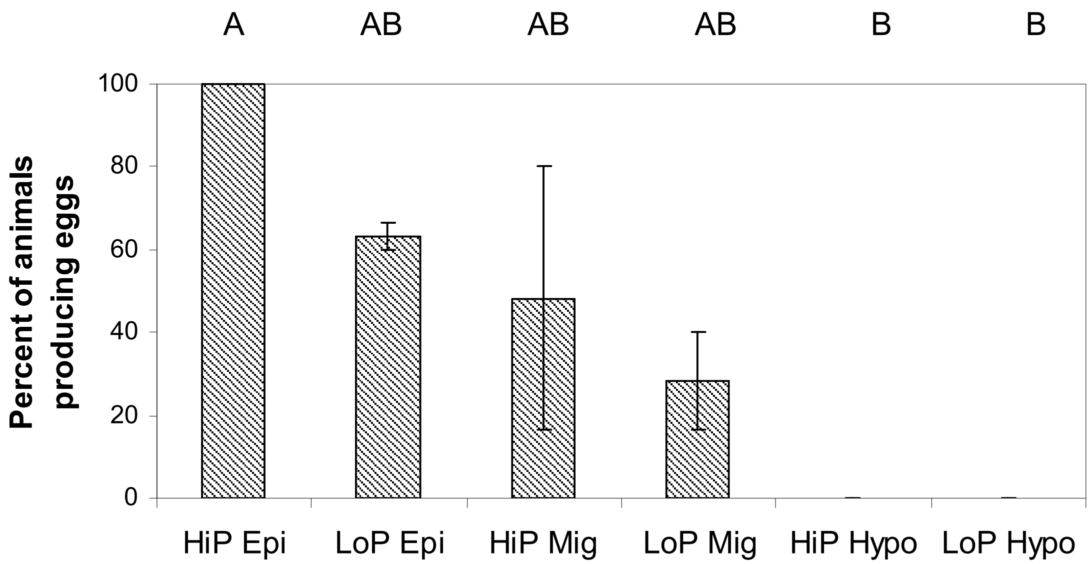

Figure 7. Percentage of *Daphnia* in each of the six restriction treatments that produced eggs (mean ± SE, n = 2 tubes per treatment). Multiple comparisons among means using Tukey's HSD ($\alpha = 0.05$) indicated differences among means as noted by uncommon letters above bars.

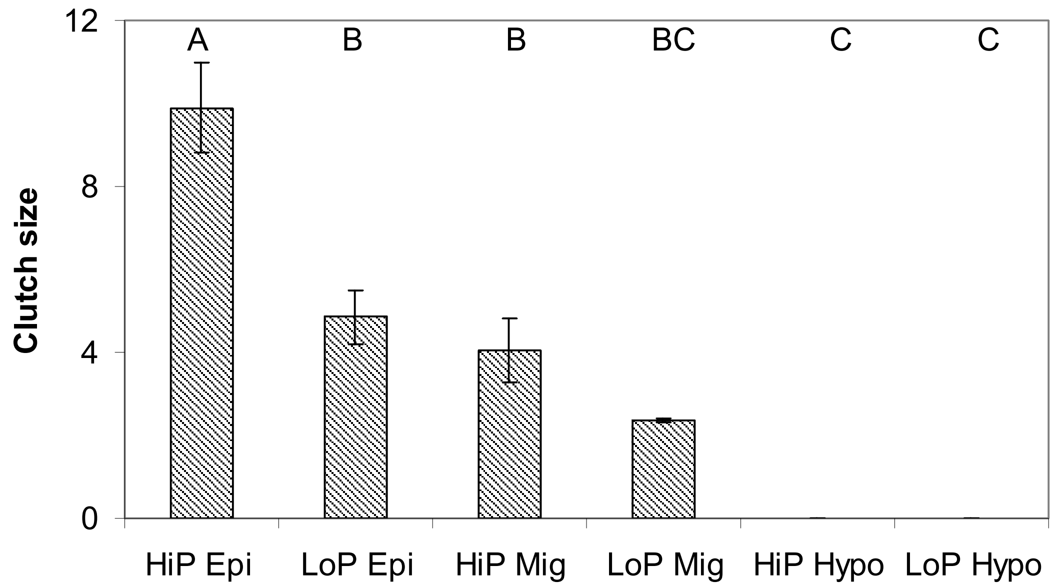

Figure 8. Clutch size per individual *Daphnia* in restriction study (mean ± SE, n = 2 tubes per treatment). Multiple comparisons among means using Tukey's HSD ($\alpha = 0.05$) indicated differences among means (as noted by uncommon letters above bars).

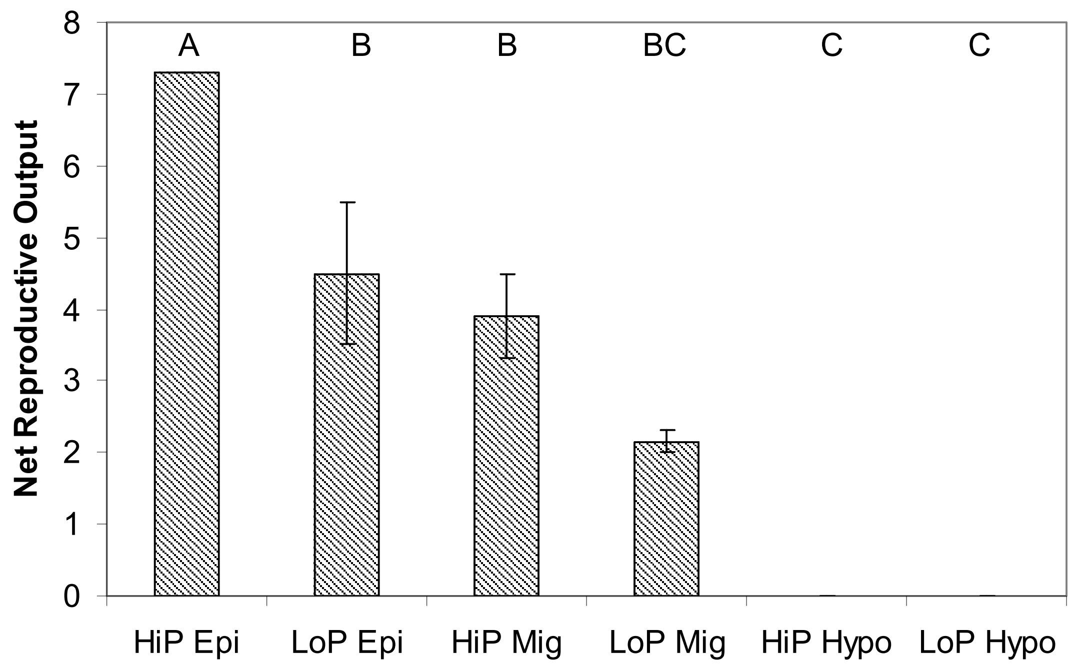

Figure 9. Net reproductive output of *Daphnia* in restriction study (mean ± SE, n = 2 tubes per treatment). Multiple comparisons among means using Tukey's HSD indicated differences among means (as noted by uncommon letters above bars).

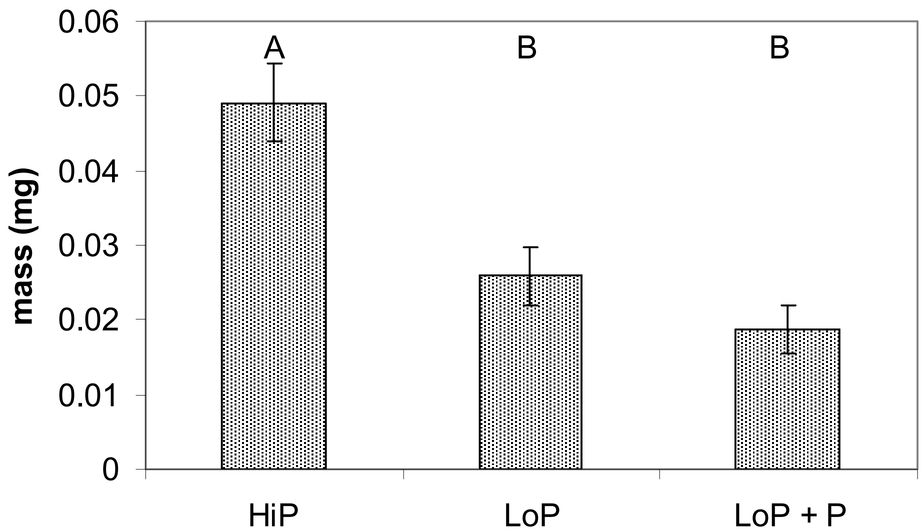

Figure 10. Final dry mass of *Daphnia* in the phosphorus addition experiment (mean ± SE, n = 4 tubes of HiP and LoP treatments and 3 tubes of LoP + P treatment.) Vertically homogeneous high food quality is represented by HiP; vertically homogeneous low food quality is represented by LoP. LoP + P represents low-quality food in the epilimnion and high-quality food in the hypolimnion. Multiple comparisons among means using Tukey's HSD indicated differences among means (as noted by different letters above bars).

Chapter 4

A field study of *Daphnia* daytime position within 34 thermally-stratified upper Midwestern lakes

Abstract

Traditional diel vertical migration (DVM) theory predicts that zooplankton will spend the daytime in the cool, dark, hypolimnetic waters of a lake in an effort to avoid predation by visually-hunting predators. Laboratory studies (see Chapter 2) indicate that mature *Daphnia* may maximize their fitness by remaining in the epilimnion (despite increased predation risk) when food quality (in terms of phosphorus) is high. However, when food quality is low in the epilimnion, adult *Daphnia* have been shown to remain within the hypolimnion. The advantage of this retreat is three-fold. First, the cool temperatures of the hypolimnion reduce the metabolic demands on the organism. Second, predation pressure in the hypolimnion is relaxed, and third, the hypolimnion frequently contains food of higher nutritive quality than the epilimnion. This study used data collected from natural environments to determine if *Daphnia* are distributed similarly with respect to food quality in natural lakes as they were in the laboratory. Data were analyzed using chemical and biological data collected from 35 thermally-stratified lakes that were

sampled in the Upper Midwest, U.S.A. I found that, when food quality was good throughout the water column, *Daphnia* spp. densities were higher in the warm, upper layer. In contrast, when food quality was good in the lower layer and poor in the upper layer, no significant lake-layer density difference was noted among the *Daphnia* species. Using species-specific analyses, *D. retrocurva* and *D. mendotae* exhibited a clear preference for the upper lake layer when food quality was good throughout the water column, but not when food quality was poor in the upper layer. *D. pulicaria* did not exhibit any layer preference in either lake environment.

Introduction

Habitat selection has long been an area of ecological study. For limnologists, the question has long been, "*How do Daphnia choose where in the water column to reside?"* Traditional diel vertical migration (DVM) theory states that zooplankton spend the daytime in the cool, dark, hypolimnion in order to avoid visually-hunting predators, and that they rise into the warmer epilimnion during the nighttime to feed. While previous research assumed the epilimnion to be very food-rich (McLaren 1974; Zaret and Suffern 1976; Enright 1977; Stich and Lampert 1981; Guisande et al. 1991), it has since been argued that epilimnetic algae are often lower in abundance (Williamson et al. 1996) and/or nutritive quality (Sterner et al. 1997) than that found in the hypolimnion and that algal quality might be better in deeper waters.

This finding, that the lower, cooler lake layers may contain higher quality (in terms of P) algae than the epilimnion, requires a shift in the way ecologists view DVM. In a stratified lake, if an animal remains in the warmer epilimnion, assuming it is able to survive predation by visually-hunting planktivores, it will experience an increased growth rate due to the warmer temperatures. If the animal resides in the hypolimnion during the daytime, its growth is constrained by cool temperatures, but it faces reduced predation pressure. However, habitat or strata selection likely involves more factors than simply temperature and predation pressure. Other characteristics, including the potential for oxygen limitation in the hypolimnion and algal nutritive quality within each lake stratum must be considered. There must be a balance among multiple factors associated with the vertical gradient that help zooplankton realize maximum productivity, and thus maximum fitness, in the presence of visually-hunting predators.

While studies of laboratory and field zooplankton DVM have generally focused on the roles of single factors in determining or altering animal daytime behavior [e.g., predators (Dawidowicz et al. 1990), light (Van Gool and Ringelberg 2003; Ringelberg 1999; Winder and Spaak 2002; Hanazato et al. 2002), and food quantity, (Pijanowska and Dawidowicz 1987; Gliwicz and Pijanowska 1988)], few studies have examined the C:P ratio of various lake layers using a large-scale survey (but see MacKay 1996 and Jeppesen et al. 2003). In addition, few studies have examined optimal foraging behavior of zooplankton on a large scale while considering multiple potential main effects (but see Jeppesen et al. 2003). Laboratory studies conducted as a part of this dissertation have demonstrated that mature *Daphnia* alter their daytime vertical position in a column of water in response to variations in food quality, location of algal maxima, and presence or absence of predator chemical cues (see Chapter 2 of this dissertation). The focus of this chapter is to determine if *Daphnia* are distributed in a similar fashion with respect to food quality in natural environments.

In this chapter I examine a snapshot of the chemical and biological parameters of 34 stratified lakes throughout the upper Midwest (Minnesota, Iowa, and Michigan), U.S.A. during the summers of either 2000 or 2001. I predicted that *Daphnia* would avoid strata with high C:P algae (i.e., poor-quality algae) and would instead reside in the more nutrient-rich layers, if available. In addition, I predicted that *Daphnia* would risk both predation and increased metabolic demands (due to the increase in temperature) to remain in the upper layer during the daytime only when food quality in the upper layer was high.

Methods

A total of 128 lakes and ponds located in six different regions throughout the upper Midwest, U.S.A. (Northeast (lakes near Ely), Northwest (lakes near Itasca), and the Twin Cities metro area in Minnesota, Michigan, Iowa, and South Dakota (Fig. 1)) were selected and sampled once during the daytime during the summers of either 2000 or 2001. Sampling was conducted by graduate students from Dr. James Cotner's and Dr. Robert Sterner's laboratories at the University of Minnesota. All lakes were sampled either at the deepest point or, if the deepest point was not known, in the center of the lake. Temperature and oxygen profiles were created using a hand-held YSI meter, and if the lake was stratified, the boundaries of the epilimnion, metalimnion, and hypolimnion were determined using the temperature profile. Thirty-five of the 128 lakes were found to be thermally stratified, and this chapter focuses exclusively on those stratified lakes. See Tables 1 and 2 in Appendix C for selected physical and chemical parameters pertaining to each lake.

Daphnia have been shown to have little to no algal cell selective ability (Bogdan and McNaught 1975, McNaught et al. 1980, Butler et al. 1989, Bern 1994); they are filter feeders and consume what fits into their mouths. To examine the stoichiometry of the *Daphnia* food within each lake stratum (epilimnion, metalimnion, and hypolimnion), seston (defined as suspended particulate matter including algae, bacteria, detritus, and inorganic material) was sampled in duplicate from two locations within each stratum using a 4-liter Van Dorn sampler. In order to obtain the size fraction of seston that *Daphnia* are able to consume, water from the Van Dorn sampler was filtered through an 80-µm mesh filter on the boat, transferred into 20-liter carboys, and taken back to the lab for additional filtrations. All samples were kept in the dark in a large cooler during transport between the lake and the laboratory, and all samples were processed and/or preserved within six hours of being removed from the lake (Hall 2006).

In order to quantify zooplankton assemblages, duplicate samples were taken in each lake at the same depths and locations as the seston samples described above. Zooplankton were sampled using a 15-liter Schindler-Patalas trap, except for Lake Itasca, where zooplankton were sampled using a 4-liter Van Dorn sampler (reasoning unknown). The contents of the zooplankton sampler were emptied into 200-ml bottles, stored in a cooler, and returned to the laboratory. All samples were preserved within six hours of being removed from the lake (Hall 2006).

In the laboratory, duplicate samples (500 ml) of the seston-containing filtered water were filtered onto precombusted GF/F filters for seston carbon analysis and onto two precombusted, acid-washed GF/F filters for seston phosphorus determination. All filters were placed in Teflon®-lined petri dishes, dried overnight at 60°C, placed in glassine envelopes, and then stored in desiccators until analysis. Near-infrared spectroscopy (Foss NIRS-5000) was used in order to determine seston C (Hood et al. 2006). Particulate P was determined by the ascorbate-reduced molybdenum-blue method after digestion with potassium persulfate at 120°C for 30 minutes (American Public Health Association 1992). Seston C:P was determined by comparing the atomic ratio of carbon to phosphorus.

Zooplankton samples were split as needed, preserved with Lugol's solution, and stored in Nalgene® bottles at 4°C until enumerated. To determine zooplankton abundance, three 1-ml subsamples were taken using a Hensen-Stempel pipet, placed into a Sedgewick-Rafter chamber, and counted using a dissecting microscope. Each subsample result was then converted to number of animals per liter.

In order to remain consistent with experimental procedures from Chapter 2 of this dissertation, epilimnetic and metalimnetic data were merged to form one "upper" layer. The following equation was used in order to obtain the upper layer density of zooplankton in each lake:

$$\frac{(\rho_{epi} * \delta_{epi}) + (\rho_{meta} * \delta_{meta})}{(\delta_{epi} + \delta_{meta})}$$

where ρ represents the density of *Daphnia* in a layer and δ represents the thickness of a layer. No conversions were necessary for the hypolimnetic data, though hypolimnetic densities are hereafter called "lower" layer densities (to be consistent with the "upper/lower" layer terminology).

When comparing the percent of the *Daphnia* density in each layer among the lakes with fish abundance and dissolved oxygen content, upper layer percentages were determined using the following equation:

$$\frac{\rho_{upper}}{\rho_{lower} + \rho_{upper}} * 100$$

where ρ_{upper} represents the density of *Daphnia* in the upper layer ρ_{lower} represents the density of *Daphnia* in the lower layer. Conversely, the percent of the density of animals found in the lower layers were determined using the following equation:

$$\frac{\rho_{lower}}{\rho_{lower} + \rho_{upper}} * 100$$

The conversion to percentages was necessary in order to account for the large lake-to-lake variation in densities.

Fish data from routine fisheries surveys were obtained from the Michigan Department of Natural Resources, the Iowa Department of Natural Resources, and the Minnesota Department of Natural Resources. Gill net survey data was obtained for the year closest to the 2000 and 2001 (though sampling occurred between 1998 to 2005). The number of gill net samples taken in each lake varied among the lakes and ranged from one to 24 nets per lake. Survey data included black and white crappie (*Pomoxis nigromaculatus* and *Pomoxis annularis*, respectively), bluegills (*Lepomis macrochirus*), and yellow perch (*Perca flavescens*) counts (fish per gill net), and the counts were added together to represent the abundance of planktivorous fish per lake. No fish data were obtained for 16 Lake, Crispell Lake, or Miner Lake. See Table 3 in Appendix C for sampling year, number of net samples taken, and fish abundance for each lake.

Daytime spatial locations of animals were statistically analyzed using paired t-tests of the mean densities of *Daphnia* in the upper and lower layers to determine if they differed. Actual densities (± one standard error) of each layer are presented graphically. Simple linear regressions were performed to examine the relationship between planktivorous fish and *Daphnia* percent in the upper layer. Simple linear regressions were also performed to examine the relationship between percentage of *Daphnia* in the lower layer and lower layer dissolved oxygen content. All statistics were performed using Excel 2002.

Results

Daphnia spp. were identified in 34 of the 35 stratified lakes. No *Daphnia* were detected in Barton Lake; thus, this lake was excluded from further analysis. Four species of *Daphnia* were identified in the 34 lakes: *D. ambigua,* the smallest species, which is less than 1 mm in size (Ward and Whipple 1959); *D. retrocurva,* which measures up to 1.6 mm (Ward and Whipple 1959); *D. mendotae,* which measures between 1.3 and 3.0 mm (Ward and Whipple 1959); and *D. pulicaria*, the largest of the species, which can measure up to 3.0 mm in length (Wetzel 2001). See Tables 4A through 4D in Appendix C for densities of *Daphnia* species detected in each lake. Only two of the lakes (Okoboji and Little Sand) contained identified populations of *D. ambigua*; therefore, species-specific analyses (*Daphnia* percentage vs. dissolved oxygen concentration in the lower layer, and percent *Daphnia* in the upper layer vs. planktivorous fish abundance) were not performed on those populations. *D. retrocurva* were identified in 11 lakes, and *D. mendotae* and *D. pulicaria* were each identified in 22 lakes.

Seston C:P was under 300 (hence considered good-quality food as defined by Urabe et al. 1997) in the lower layer of all 34 lakes. Four of the lakes (Green, 16 Lake, Lee, and Derby) had relatively high seston C:P ratios in the upper layer (C:P of 321, 498, 520, and 570 respectively), while the other 30 lakes had upper layer seston C:P ratios less than 300.

Daphnia spp. daytime location was affected by the nutritive quality of the upper lake layer. *Daphnia* densities were equal in the upper and lower layers when C:P in the upper lake layer was high, (5.98 ± 4.46 animals/liter in the upper layer vs. 7.68 ± 2.66 animals/liter in the lower layer, $t(3) = -0.65$, $p = 0.28$). This result was likely not significant likely due to low sample size and/or the large standard error. Conversely, when C:P in the upper layer was low, the density of *Daphnia* was higher in the warmer upper layer (7.21 ± 1.29 animals/liter in the upper layer vs. 4.75 ± 0.77 animals/liter in the lower layer, $t(3) = 2.74$, $p = 0.0051$) (Fig. 2).

Among the 30 lakes with good-quality food throughout the water column, the degree of upper layer preference varied among the different *Daphnia* species (Fig. 3A). The densities of *D. retrocurva* and *D. mendotae* found in the upper lake layers were significantly greater than the densities found in the lower layers (5.62 ± 1.73 animals/liter in the upper layer vs. 2.48 ± 0.94 animals/liter in the lower layer, $t(9) = 2.43$, $p = 0.019$ and 6.13 ± 0.39 animals/liter in the upper layer vs. 3.28 ± 0.21 animals/liter in the lower layer, $t(19) = 2.12$, $p = 0.023$ respectively). *D. pulicaria* were equally likely to be found in either layer (1.72 ± 0.57 animals/liter in the upper layer vs. 2.62 ± 0.82 animals/liter in the lower layer; $t(17) = -1.14$, $p = 0.13$).

Among the four lakes with poor-quality food in the upper layer, there was no significant layer preference among any of the *Daphnia* species (Fig. 3B). Only one lake (Green Lake) contained *D. retrocurva* populations; in that lake, approximately 57% of the density of the *D. retrocurva* population were found in the upper layer. Two lakes (Green Lake and Lee Lake) contained populations of *D. mendotae*, and all four lakes contained populations of *D. pulicaria*. *D. mendotae* and *D. pulicaria* were equally likely to be found in either lake layer (0.9 ± 0.9 animals/liter in the upper layer vs. 1.42 ± 0.092

animals/liter in the lower layer; $t(1) = -0.55$, $p = 0.34$ and 1.25 ± 0.64 animals/liter in the upper layer and 3.73 ± 1.69 animals/liter in the lower layer; $t(3) = -1.73$, $p = 0.091$) respectively.

Among lakes with good-quality food throughout the water column, upper layer *D. pulicaria* populations did not appear to be significantly affected by the planktivore abundance in the lake ($y = -0.21x + 56.472$, $R^2 = 0.040$, $p = 0.46$, Fig. 4A, Table 1). In order to determine if the two lakes with the highest planktivore abundance were obscuring the relationship, those lakes (Minnesota's Long Lake and Lake Itasca) were removed from the analysis, and a second regression was performed. In this case, planktivore abundance still did not appear to be driving *D. pulicaria* from the upper lake layers during the daytime, and in fact there was a positive relationship between the percentage of *D. pulicaria* in the upper layer and fish density ($y = 1.66x + 28.67$, $R^2 = 0.35$, $p = 0.025$, Fig. 4B, Table 1).

Among lakes with high upper layer C:P, no relationship was detected between planktivorous fish and the percentage of the density of *Daphnia* spp in the upper layer ($y = 1.36x - 0.45$, $R^2 = 0.79$, $p = 0.31$, Fig. 4C, Table 2).

Invertebrate predators were rarely found in the samples. One *Leptidora* was counted in a hypolimnetic sample from Bear Island. *Chaoborus* were slightly more common, with one animal counted in a hypolimnetic sample from Burntside Lake, one in an epilimnetic sample from Lake Plantagenet, one in a metalimnetic sample and one in a hypolimnetic sample from Green Lake, one in a metalimnetic sample from Barton Lake, and one counted in a hypolimnetic sample from Lee Lake.

A significantly larger percentage of the density of *D. retrocurva* and *D. mendotae* were found in upper lake layers. Interestingly, nine of the ten lakes containing *D. retrocurva* populations and good-quality food throughout the water column had low dissolved oxygen (DO) content in the lower layer (defined as < 3.5 mg/l according to Winder et al. 2003). However, low DO did not appear to be driving the *D. retrocurva* into the upper layer as no significant relationship between lower layer dissolved oxygen content among the low DO lakes and percentage of *D. retrocurva* in the lower layer was detected when a regression was performed on the two variables ($y = -36.92x + 40.48$, $R^2 = 0.17$, $p = 0.27$, Fig. 5A, Table 3). Examination of the data revealed a trend of decreasing percentages of the density of *D. retrocurva* in the lower layer as DO declines in lakes with very low DO concentrations. When lakes with DO greater than 0.2 mg/l were excluded from the analysis, the relationship between the proportion of the density of *D. retrocurva* found in the lower layer and the DO content was significant ($y = 512.08x - 31.599$, $R^2 = 0.596$, $p = 0.042$, Fig. 5B, Table 3). While it should be noted that this range of DO content is very small, and thus possibly not a truly meaningful range for a trend, in these lakes, when DO is less than 0.2 mg/l, there is a positive relationship between the occurrence of *D. retrocurva* in the lower layer and lower layer DO.

Among lakes with low DO concentrations in the lower layer, the relationship between the percentage of *D. mendotae* found in the lower layer and lower layer DO was not significant ($y = 29.50x + 19.67$, $R^2 = 0.11$, $p = 0.3$, Fig. 6, Table 4). Low dissolved oxygen content in the lower layer was not forcing *D. mendotae* to remain in the upper layers during the daytime.

Discussion

The warm epilimnion of a lake is generally a well-lit environment; thus, the conventional wisdom is that zooplankton favor lower lake layers during the daytime in order to avoid visually-hunting predators (e.g., Zaret and Suffern 1976). These lower layers are darker and thus provide refuge from fish and other visually-hunting predators. In addition, it had previously been thought that the upper layers of lakes contain the "best" algae (in terms of nutritional quality) (see references in MacKay 1996; Gliwicz 2003). More recent studies, however, indicate that the lower lake layers are likely to contain more abundant (Williamson et al. 1996; Winder et al. 2003) and nutritionally superior (Sterner et al. 1997) algae. The reason for this is as follows: Since light enters a lake at the surface and nutrients generally arise from the sediments, mixing must occur for the hypolimnetic nutrients to reach the epilimnion. During midsummer stratification, sufficient quantities of hypolimnetic nutrients are unable to be mixed into the epilimnion. When light penetrates to lower layers of the lake, metalimnetic and hypolimnetic seston are able to take up these nutrients and produce high quality food. In contrast, the epilimnion, with its abundant light but fewer nutrients, produces more carbon-rich, lower quality food. Therefore, the algae in the lower layer is frequently of higher nutritive quality (and with a lower C:P ratio) than epilimnetic algae. Thus, when choosing where to reside during the daytime, zooplankton experience a trade-off between, among other factors, the predation risk in the upper layer's well-lit waters and the refuge of the dark, though nutrient-rich, lower layer.

When the upper layer is of high nutritive quality (defined as C:P < 300), it is not surprising that *Daphnia* might risk predation in order to reside in its warm, nutrient-rich water. The warmer temperatures of the upper layer are positively correlated with increased growth rates, which in turn are associated with a decrease in generation time (Giebelhausen and Lampert 2001) and, theoretically, an increase in population size. However, as the nutritional quality of the upper layer declines, a corresponding shift in the distribution of *Daphnia* throughout the vertical profile should occur.

In this field study, despite the known presence of planktivorous fish in the majority of the lakes, when presented with high food quality in the upper lake layers, *Daphnia* spp. were significantly more likely to reside in the warmer, nutrient-rich waters despite the increased risk of predation. In contrast, when the C:P was high in the upper lake layer, *Daphnia* spp. showed no preference in habitat (Figure 2).

Among those lakes with good-quality food throughout the water column, when the three most abundant species were examined individually, *D. retrocurva* and *D. mendotae* exhibited markedly different responses in habitat selection than *D. pulicaria*. *D. retrocurva* and *D. mendotae* densities were higher in the warm surface layer (as opposed to the cooler lower layer) when food quality in the upper layer was good (Figure 3A).

In the lakes with low C:P throughout the water column, *D. pulicaria* densities were similar in the lower and upper layers (Figure 3A). The possibility exists that planktivorous fish predation was driving the *D. pulicaria* populations into lower lake layers. This, however, was not found to be the case. While there appeared to be a negative trend between the abundance of planktivores and *D. pulicaria* density in the upper layer, when lakes with high planktivore abundance were removed, this apparent (though not significant) negative trend was no longer apparent. Thus we are able to reject the hypothesis that planktivorous fish are creating a hostile upper-layer environment for *D. pulicaria* (Figure 4A and 4B).

Why would *D. retrocurva* and *D. mendotae* exploit the nutrient-rich upper layer during the daytime, while *D. pulicaria* would not? In many temperate lakes, especially later in the summertime, hypolimnetic waters may exhibit anoxic or hypoxic conditions. Weider and Lampert (1985) noted that at a critical oxygen concentration of 3.5 mg/l, filtering and respiration rates of *D. pulex* decreased dramatically. Survivorship of the animals did not appear to suffer; rather decreases in filtering and respiration rates have been postulated to impact life-history traits such as juvenile growth rate (Hanazato 1995) and overall reproductive output per individual.

Examination of the lakes where *D. retrocurva* were abundant revealed that nine of the ten high-P lakes had low oxygen levels in the hypolimnion. Individuals were detected in the lower layer of all but one of the hypoxic lakes. However, there was a negative relationship between the percentage of *D. retrocurva* in the lower layer and lower layer DO when the DO content was below 0.2 mg/l (Figure 5B). This relationship indicates that hypoxia may have been a driving factor in habitat selection for *D. retrocurva* (at least when DO was < 0.2 mg/l). It should also be noted, however, that the inhospitable lower layer may *not* have been a factor in habitat selection. The possibility exists that *D. retrocurva* chose to inhabit the upper layer solely due to its warm and nutrient-rich environment. Interestingly, the three lakes with DO > 0.2 mg/l in the lower layer (0.6, 1.0, and 6.1 mg/l) also had very low percentages of *D. retrocurva* in the lower layers (14.4%, 0%, and 0% of the total density within each lake respectively).

While the majority of the above-referenced lakes had populations of *D. mendotae*, no associated positive correlation was detected between *D. mendotae* lower layer density and lower layer DO content. Thus, low DO content was likely not the driving factor in *D. mendotae* upper layer preference (Figure 6).

While more *D. retrocurva* and *D. mendotae* resided in the upper lake layers when food quality within the layer was high, when food quality within the upper layer was poor, *Daphnia* exhibited no layer preference. This finding that *Daphnia* exploited the warmer temperatures only when food quality was high is consistent with the experimental findings in Chapter 2 of this dissertation.

Among the high upper layer C:P lakes, *Daphnia* spp. were equally likely to be found in either layer. The possibility exists that those *Daphnia* spp. residing in the lower layer chose to reside in that lower layer due to its low C:P. It could also be argued that the density of *Daphnia* spp. in the upper layer was reduced due to predation pressure by planktivorous fish. However, as noted in Figure 4C, a no negative correlation was identified between the two variables. Thus, it is unlikely that predation pressure was driving *Daphnia* spp. from the upper layer, and the dispersal of animals was related to something else (perhaps food quality). It should be noted that the sample size of the lakes with high upper layer C:P was modest (four lakes) and that fish data were obtained for only three of the lakes.

But let us return to the original question: How do *Daphnia* choose what layer to inhabit during the daytime? In order to further unravel the complexities of zooplankton habitat selection, several factors should be considered for future experiments. First, sampling was conducted throughout the entire summer (May to August). Seasonal variation may explain some of the variation in the data. Secondly, while numerous lakes with low C:P throughout the water column were included in this study, future sampling efforts should include a larger percentage of lakes with high C:P in the upper layer. The inclusion of these more nutrient-poor lakes would allow for a stronger species-level assessment of the *Daphnia* habitat choice.

Furthermore, while a single sampling effort within a lake provides insight into *Daphnia* daytime distribution, Visman et al. (1994) concluded that the distribution of zooplankton can vary so greatly throughout a lake and over a period of time that population estimates derived from a single midlake station may lead to "unacceptable errors." Thus, it would be beneficial to increase the number of sampling locations and the sampling frequency in order to better understand zooplankton dispersal.

In addition, future examination of *Daphnia* habitat selection should consider the age of each *Daphnia* enumerated. Numerous studies have discussed the effects ontogeny has on zooplankton distribution [including copepods as described in Zadereev and Tolomeyev (2007) and cladocera as described in Primicerio (2003) and Lampert et al. (2003)]. A previous study (Chapter 3 of this dissertation) indicated that, in the laboratory, juvenile *Daphnia* do not actively seek out high-quality algae within a water column. Thus, it is possible that *Daphnia* ontogeny (perhaps especially among *D. pulicaria*) affects habitat selection in the field, just as it did in the laboratory experiments conducted for Chapters 2 and 3 of this dissertation.

Finally, interspecific variation exists in the C:P of zooplankton. Sterner and Elser (2002) showed that the somatic C:P of *D. pulicaria* is greater than the somatic C:P of *D. galeata* (which often hybridizes to form *D. galeata mendotae*). Was the lack of lake-layer preference among *D. pulicaria* related to the internal C:P of that species? What is the somatic C:P of *D. retrocurva*? If the C:P of *D. retrocurva* is similar to that of *D. galeata*, perhaps internal animal C:P explains the species-specific variation in habitat preference.

Although this study has increased our knowledge of *Daphnia* habitat selection, it also raises additional questions that should be examined in the future in order to more thoroughly understand their behavior.

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Table 1. Degrees of freedom, sums of squares, F, and p values from a regression of percent *D. pulicaria* in the upper layer and planktivore abundance per gill net among all low C:P lakes and among low C:P lakes with low planktivore pressure.

Factor	All low C:P lakes				Low C:P lakes with low predation pressure			
	df	*SS*	*F*	*p*	*df*	*SS*	*F*	*p*
Regression	1	1130.86	0.59	0.46	1	8237.03	6.53	**0.025***
Residual	14	26933.71			12	15126.53		
Total	15	28064.58			13	23363.56		

Significance at $^*P < 0.05$

Table 2. Degrees of freedom, sums of squares, F, and p values from a regression of percent *Daphnia* spp. in the upper layer and planktivore abundance per gill net among lakes with low C:P lower layers and high C:P upper layers.

Factor	Among lakes with high C:P upper layer			
	df	*SS*	*F*	*p*
Regression	1	1334.00	3.67	0.31
Residual	1	363.51		
Total	2	1697.51		

Table 3. Degrees of freedom, sums of squares, F, and p values from a regression of percent *D. retrocurva* in the lower layer and lower layer dissolved oxygen (DO) content when DO concentrations were less than 1.0 mg/l and less than 0.2 mg/l.

Factor	When DO is < 1.0 mg/l				When DO is < 0.2 mg/l			
	df	*SS*	*F*	*p*	*df*	*SS*	*F*	*p*
Regression	1	2697.16	7.38	**0.042***	1	1013.29	1.43	0.27
Residual	5	1828.00			7	4977.30		
Total	6	4525.15			8	5990.58		

Significance at **P* < 0.05

Table 4. Degrees of freedom, sums of squares, F, and p values from a regression of percent *D. mendotae* in the lower layer and lower layer dissolved oxygen (DO) content when DO concentrations were less than 2.0 mg/l.

Factor	When DO is < 2.0 mg/l			
	df	*SS*	*F*	*p*
Regression	1	1123.86	1.13	0.31
Residual	9	8923.80		
Total	10	10047.66		

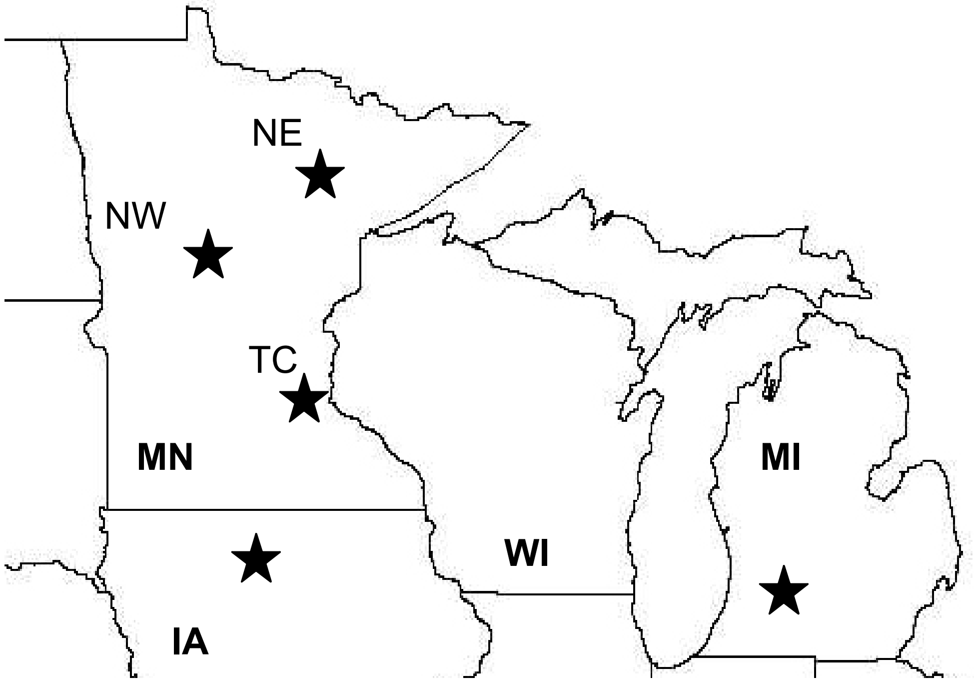


Figure 1. Map of approximate sampling sites in Minnesota, Michigan, and Iowa, USA. South Dakota sampling sites are not depicted because none of those lakes were thermally stratified, and therefore, those data were not used in subsequent analyses. Stars represent lake regions sampled.

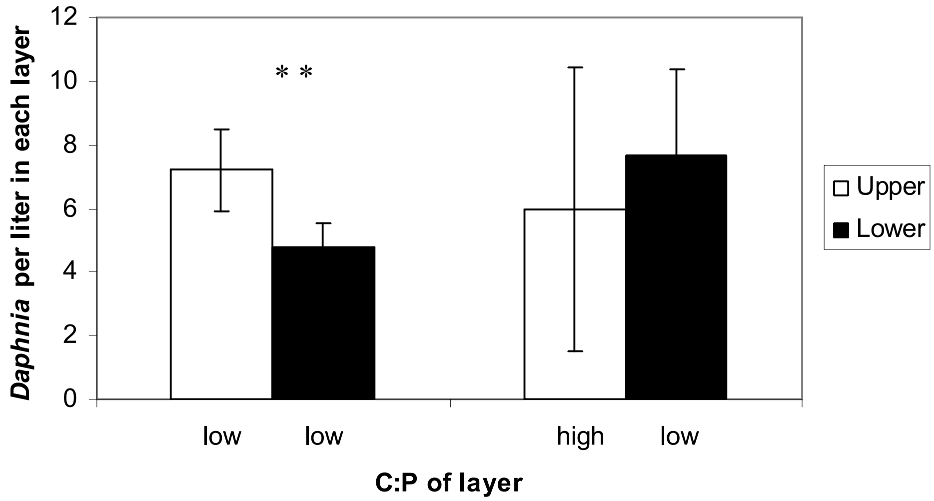


Figure 2. Mean (± 1 standard error) density of *Daphnia* spp. per liter found in upper and lower lake layers. Bars described as low C:P in both upper and lower layers correspond to lakes with good quality food (C:P < 300) throughout the entire water column. Bars described as high C:P in the upper layer and low C:P in the lower layer correspond to lakes with poor quality food (C:P > 300) in the upper layer and good quality food in the lower layer. Significance at the $\alpha = 0.01$ level based on paired t-test of densities is denoted by the double asterisks (**).

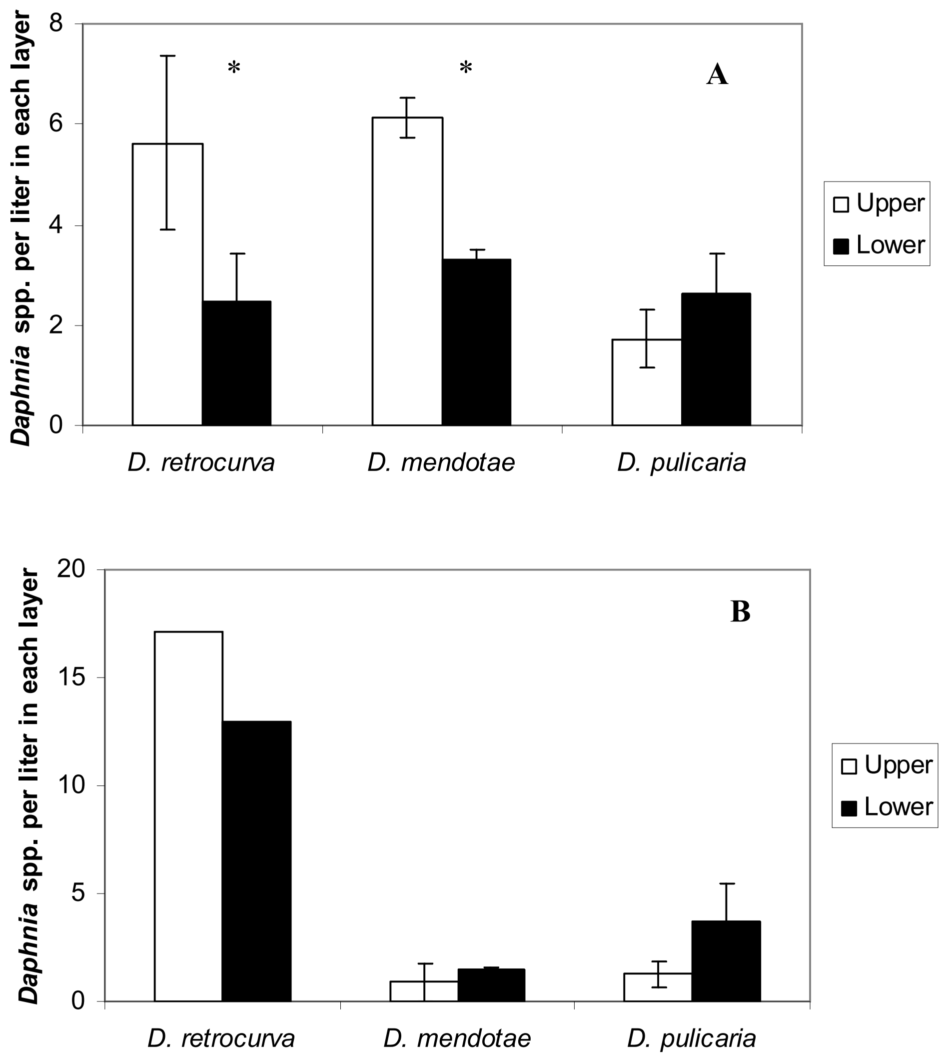


Figure 3. A. Mean density (± 1 standard error) of three *Daphnia* species found in upper and lower layers of lakes with good-quality food throughout the water column. Significance at the $\alpha = 0.05$ level based on paired t-tests of the density in each layer is denoted by the asterisk (*). B. Mean (± 1 standard error for *D. mendotae* and *D. pulicaria*) density of three *Daphnia* species found in upper and lower layers of lakes with good-quality food in the lower layer of the water column and poor-quality food in the upper layer of the water column.

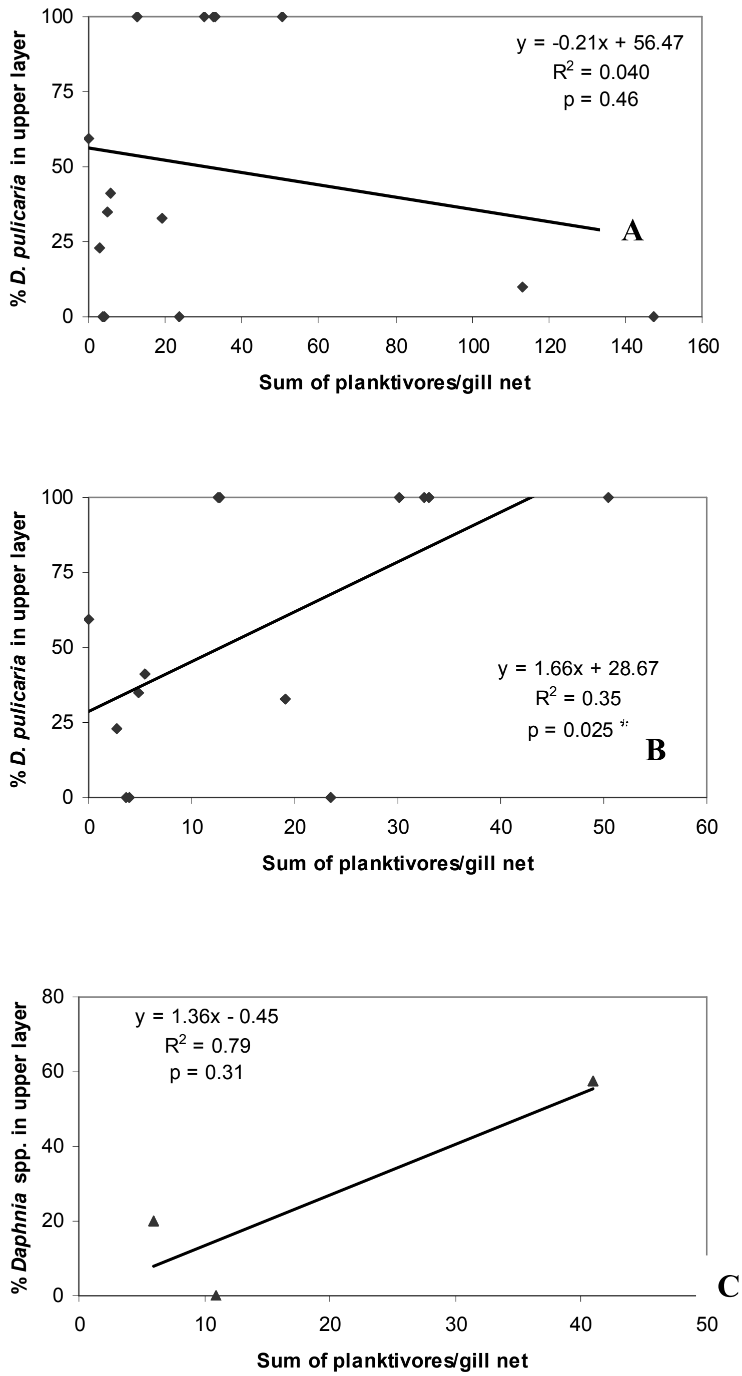
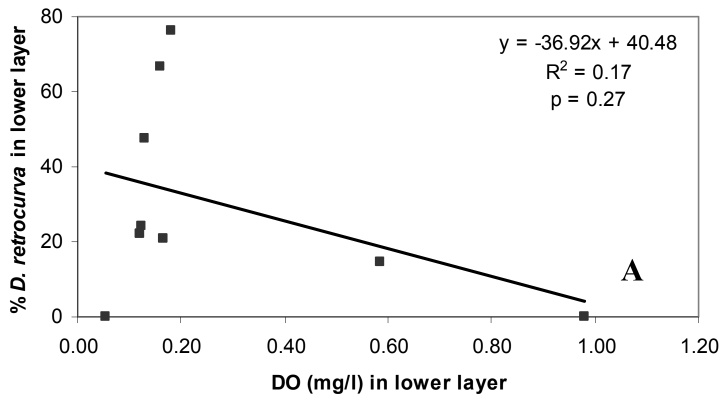


Figure 4. A. Relationship between percent of the density of *D. pulicaria* found in the upper lake layer during the daytime and the sum of planktivores per gill net in each lake

containing *D. pulicaria* populations. B. Relationship between percent of the density of *D. pulicaria* found in the upper lake layer during the daytime and the sum of planktivores per gill net in each lake containing *D. pulicaria* populations when the two high-planktivore lakes were excluded. C. Relationship between percent of the density of *Daphnia* spp. found in the upper layer during the daytime and the sum of planktivores per gill net in lakes with high C:P in the upper layers. Fish data were not available for 16 Lake, a high C:P lake. Regression lines are indicated within each group. Significance at the $\alpha = 0.05$ level is denoted by an asterisk (*). See Table 1 for regression statistics.



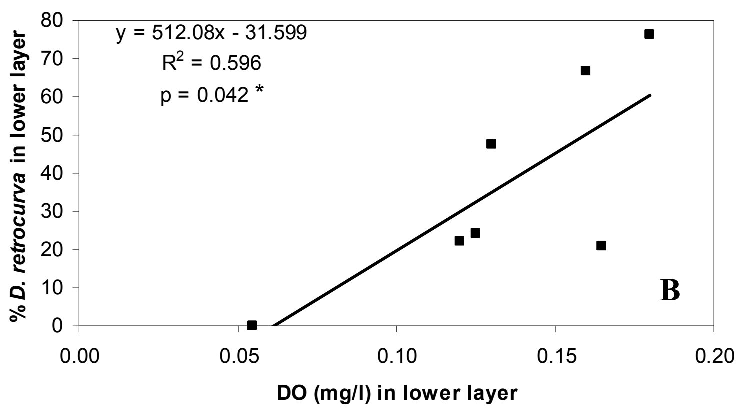


Figure 5. A. Relationship between the percent of the density of *D. retrocurva* found in the lower lake layer during the daytime and the dissolved oxygen content in the lower layer. Lakes included in this analysis contained *D. retrocurva* populations and low dissolved oxygen (< 3.5 mg/l) in the lower layer. B. Regression of the same variables as in (A), except lakes with dissolved oxygen content above 0.2 mg/l in the lower layer were excluded from the analysis. Regression lines are indicated within each group. Significance at the $\alpha = 0.05$ level is denoted by the an asterisk (*). See Table 2 for regression statistics.

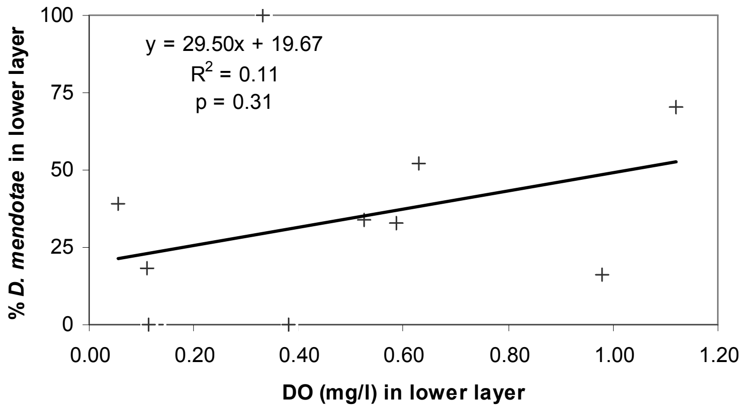


Figure 6. Relationship between the percent of the density of *D. mendotae* found in the lower lake layer during the daytime and the dissolved oxygen content in the lower layer. Lakes included in this analysis contained *D. mendotae* populations and low dissolved oxygen (< 2.0 mg/l) in the lower layer. The regression line for the data is indicated.

Chapter 5

Summary

Habitat selection has long been an area of ecological study. Diel vertical migration (DVM), zooplankton habitat selection within a vertically patchy water column over time, has been studied for over a century. Previous DVM studies have examined the effects of varying temperatures (McLaren 1963; Calaban and Makarewicz 1982; Makino and Ban 1998), light regimes (Ringelberg and Van Gool, 2003; Ringelberg 1999; Winder and Spaak 2002; Hanazato et al. 2002), predation pressures (Dawidowicz et al. 1990), ontogeny (Kessler and Lampert 2004) and food quantities (Pijanowska and Dawidowicz 1987; Gliwicz and Pijanowska 1988) on zooplankton daytime location and on migratory amplitude. Only recently, though, have researchers begun to explore the relationship between seston stoichiometry and zooplankton habitat selection (ex. MacKay 1996; Schatz and McCauley 2007; Reichwaldt 2008; and Forman Chapters 2 and 3).

While previous studies have examined the physiological responses of *Daphnia* within a static C:P environment (e.g. Sterner et al. in prep, Urabe et al. 1997) or by physically moving a daphnid between high and low quality environments (e.g. Sterner and Schwalbach 2001), with the exception of publications by Schatz and McCauley (2007) and Reichwaldt (2008), no known studies have offered a daphnid the ability to choose its own environment within a vertically-patchy water column. This study therefore affords the opportunity to examine both the behavior and the physiology of *Daphnia* in response to a variety of environments. The results of Chapter 2 demonstrated that, in a laboratory setting, mature *Daphnia* can detect variations in the nutritional content of their food, and that they alter their vertical position in response to varying phosphorus content regardless whether predator cues are present or absent. Specifically, Chapter 2 found that *Daphnia* remain low in the water column when fed low quality algae (regardless of the presence or absence of fish kairomone or food delivery location). However, when *Daphnia* were fed high quality food, migration ceased, and the animals remained near the surface of the water at all times.

Chapter 3 provided additional insight into the relationship between zooplankton behavior and seston stoichiometry by examining the effects of varying food qualities and temperatures on juvenile *Daphnia* habitat selection. During the daytime, and despite variations of food quality, neonate *Daphnia* resided near the warm epilimnion. Older juveniles fed a P-deficient diet appeared to retreat into the cooler hypolimnion as they aged, whereas juveniles fed a P-rich diet did not change their vertical position. Not surprisingly, animal mass, fecundity, and net reproductive rate were all found to be positively correlated with increased environmental temperature and dietary phosphorus. Variation in net reproductive output among juveniles fed P-rich and those fed P-poor diets differed significant, and appeared to be driven by clutch size, rather than by survivorship.

Chapter 4 provided a field-check of the laboratory results. Using a dataset of 34 thermally-stratified lakes, I found that, when food quality was good throughout the water column, *Daphnia* spp. densities were higher in the warm, upper layer. In contrast, when food quality was good in the lower layer and poor in the upper layer, no significant lake-layer density difference was noted among the *Daphnia* species. When further examined using species-specific analyses, *D. retrocurva* and *D. mendotae* exhibited a clear preference for the upper lake layer when food quality was good throughout the water column, but not when food quality was poor in the upper layer. *D. pulicaria* did not exhibit any layer preference in either lake environment.

This research provides insight into how *Daphnia* respond behaviorally and physiologically to variations in algal quality. Since lakes can vary a great deal vertically in terms of chemical and physical parameters, understanding the effects these gradients have on zooplankton behavior and life history tables is critical to understanding the phenomenon of diel vertical migration.

Numerous improvements could be made if these experiments were repeated in the future. One of the most important improvements that should be considered is the nighttime position of *Daphnia* both in the laboratory and in the field experiments. Does food quality truly affect DVM? Or does food quality only affect daytime habitat selection? Do *Daphnia* choose to remain in the hypolimnion at nighttime when food quality is poor in the epilimnion? Or do they follow the traditional model and ascend into the epilimnion even if food quality in that layer is poor? Laboratory experiments should examine *Daphnia* positions during the nighttime, and should also track their behavior during "dusk," "dawn," and "nighttime."

Field sampling should include the use of sonar to better identify aggregations of zooplankton. If *Daphnia* densities were very high on the water/sediment interface, and if the sampling equipment was not able to sample at that depth (due to the limitations of the Schindler-Patalas trap), it is possible that the lower layer *Daphnia* sampling does not provide an accurate picture of lower layer densities. Sonar images of the water column would identify large populations of *Daphnia* that are unable to be sampled through traditional methods.

The lake surveys were conducted throughout the entire summer (May through August) over two summers, and each lake was sampled on one occasion. Increasing the number of high C:P lakes and increasing the sampling frequency within each lake would be useful. Furthermore, the C:P in a lake's epilimnion tends to increase during the summer (becoming poorer quality algae). Tracking the change in the epilimnetic C:P and the spatial location of the *Daphnia* may provide additional evidence to support both the food-quality and species-specific findings identified in Chapter 4.

Finally, as was noted in Chapter 3, there is likely some critical time in which a *Daphnia* begins to respond spatially to variations in food quality. Tracking juveniles for a longer period of time (i.e. until adulthood and through reproduction) may elucidate the time and/or size in which juvenile *Daphnia* behavior shifts towards that of adults.

Future directions

Because lakes are vertically patchy, and since *Daphnia* are consumed by a variety of predators, it is intuitive that population dynamics could vary dramatically depending on the nutrient status throughout a water column. If an organism is nutrient-limited, the effects of habitat-seeking may be exaggerated (e.g. Gliwicz and Maszczyk 2006). Caswell (2001) showed through studies of life-history tables that effects of stoichiometric constraints on an individual are linked to population demography changes. Furthermore, Lagergren et al. (2008) demonstrated that vertical migration patterns vary seasonally. It would be interesting and informative to track population demography seasonally through a variety of lake types and zooplankton species. Furthermore, because lakes are open systems, it is likely that the changes in zooplankton demography (based on stoichiometry) will also affect consumer demography. One might then ask: *How might changes in algal stoichiometry directly and/or indirectly affect the behavior and physiology of predators?* (Moe et al. 2005).

Habitat selection by *Daphnia* can be further examined using an evolutionary framework. Evolutionarily, *Daphnia* should be found where their fitness is highest. In lake layers with low C:P (high quality food), *Daphnia* are able to graze on the most nutritious food in the lake. However, food quality is not the only factor that determines the suitability of a habitat. Temperature, light, food quantity, and predator presence/absence must also be considered. An evolutionary stoichiometric framework should consider not only these trade-offs, but the magnitude of these trade-offs, which may depend, in part, on the compositional similarity between the organism and environment (Kay et al. 2005). Is a daphnid more or less likely to inhabit a high food quality layer if the *Daphnia* itself is very low C:P and can grow at a maximal rate in the layer that is not viewed as the most favorable? This trade-off could also be examined by considering species with higher and lower somatic C:P values. Future studies should consider the internal C:P of a variety of zooplankton species as well as a broader range of C:P values, and variations in the steepness of the temperature gradient.

Genetics and molecular biology provide additional useful frameworks for examining *Daphnia* habitat selection in response to food quality. King and Miracle's (1995) study of *D. longispina* in a Spanish lake indicated that single populations within a lake may be subdivided into groups with different DVM patterns. Is nutrient detection a component that differs among groups with different DVM strategies? Are certain *Daphnia* species better able to detect variations in nutrient status? Are certain clones within a species better able to detect these variations? If variations exist in the nutrient detection ability of *Daphnia,* how might this affect an organisms' allocation of energy? It is known that different species of *Daphnia* allocate resources differently (Caceres and Tessier 2004). Would a species that is highly sensitive to variations in C:P that selects a habitat based on maximizing its fitness allocate resources differently than a species that is less sensitive to C:P variations? What proportion of body nutrients and energy would be allocated to growth? To reproduction? To ephippial egg production? What implications might these variations have on the genetic make-up of the population or community as a whole?

Thus, it is easy to see that we are only scratching the surface in the understanding of the relationship between habitat selection and ecological stoichiometry. Future studies have the capacity to provide a greater understanding of the role of nutrients in *Daphnia* behavior and physiology.

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Appendix A

Construction and maintenance of a thermally stratified aquarium to examine *Daphnia* vertical migration

The original thermally stratified aquarium (hereafter referred to as a plankton organ) used to study vertical migration was created by Piotr Dawidowicz at the Max Planck Institute for Limnology in Plön, Germany. When designing and building the plankton organ at the University of Minnesota, only two other plankton organs were known to exist in the world: one at the University of Konstantz, Germany (maintained by Eric Von Elert), and one at the University of Warsaw, Poland (maintained by Piotr Dawidowicz.) While some details of the plankton organ's structure and function have been published (ex. Dawidowicz and Loose, 1992a, 1992b), much of the necessary information needed to create a plankton organ with vertically migrating *Daphnia* was not published. After 12 months of unsuccessfully trying to stimulate *Daphnia* to migrate, in July 2001, I visited the University of Warsaw and I spent 3 weeks studying under P. Dawidowicz. The following is information I collected from trial and error, from examining the Polish plankton organ, and from extensive conversations/correspondence with P. Dawidowicz.

Construction of the tank

The size of the tank is not important, however, it is generally best to use a rectangular shaped tank rather than a square tank, as tubes are lined up next to each other, and not in front of or behind one another. The Univ. of MN tank is nearly 1 meter in height, while the Polish tank is less than 2 feet high. Glass or plexiglass may be used. I have not found in necessary to use any insulation around the tank itself. It is very important to remember to place a water outlet at the bottom of the tank; while it may sound obvious, forgetting this very important feature requires the user to siphon water out of the tank.

Maintenance of thermal gradient

In order to thermally stratify the tank, a chilling coil must be placed at the bottom of the tank. This feature should be a closed-loop arrangement, with water circulating from a chiller, through the plankton organ chilling coil, and back to the chiller. A metal with high heat conductivity, such as steel or aluminum should be used. Avoid the use of any metal that will release particulates into the tank after a significant amount of time submerged.

The chilling coil may be arranged either flat on the bottom of the plankton organ, or wrapped around the sides and back (vertically) of the tank. I prefer the vertical arrangement since it seems to provide a more stable thermal gradient. Theoretically, the gradient can range from room temperature at the top, to 4°C at the bottom of the tank.

If the chilling coil runs along the bottom of the organ, it is likely that a pump will be necessary to move water from directly above the coil and reintroduce it at the desired depth of the thermocline. I have used a peristaltic pump with glass rods on the ends of the tubing to remove and replace the cool water. It should be noted that Masterflex or equally durable tubing should be used since the pump is run continuously for several days and weaker tubing will crack and leak.

Plankton organ tubes

Square or round tubes may be used as individual experimental containers in the plankton organ. Nitex mesh should be placed on the bottom of the tube, and held in place either with a rubber band on the outside of the tube, or with a rubber insert on the inside of the tube. I prefer round tubes to square tubes, as one can place a rubber stopper in the top of a round tube, remove the tube and its contents from the water bath, and empty the contents into a beaker for study. Square tubes generally require the user to gently lift the tube out of the water bath (thus emptying the tube of its liquid and disrupting the thermocline) and then "rinse" the animals off the mesh.

Lighting

For the U of MN plankton organ, a light bank consisting of two 40-watt cool white fluorescent tubes hanging 0.5 meters above the tank on a 11:13 light:dark schedule provided light. In Poland, two round 20 W fluorescent bulbs 21.5 cm above the tank on a 16:8 light:dark schedule provided light (though they have also successfully used halogen light). Black paper (lined with aluminum foil) was used to shield the light bank and to ensure proper directionality and minimize scattering. Specifically, heavy black construction paper was placed over the light bank (which is suspended from the ceiling) and fell to approximately one inch below the top of the plankton organ. Side and front light shields were also used, and the back of the plankton organ was covered in black felt (also to make observations of the animals easier.) (See Table 1 for light levels). Before the light shield was used, *Daphnia* did not vertically migrate. The light bank may be placed on a timer with desired day and nighttime lengths, or on a dimmer, to simulate dusk and dawn as well as day and nighttime. In addition, a nightlight with a LED sensor was used at nighttime to simulate moonlight. This night light was found to be necessary in order for *Daphnia* to rise to the surface of the plankton organ during the nighttime.

Much has been written about the insensitivity of zooplankton to red light (Novales Flamarique and Browman, 2000), however, I noted obvious changes in zooplankton behavior when the red light was used (primarily *Daphnia* rapidly "circling.") Therefore, my nighttime observations were limited to one observation during the final night of each experiment.

<u>Filling the tank and introduction of media</u>

The plankton organ should be filled with either filtered lake water, or a media (such as COMBO as found in Kilham et al., 1998). If the tubes are filled with a specific media, then the organ as a whole should be filled with this media. One should not, for example, fill the organ with filtered lake water, but then use COMBO media in the tubes, since osmosis will occur.

Media and/or food may be introduced using one of two techniques. One may either replace the entire volume of the tube through a rapid turnover method (i.e. fill a graduated cylinder with the appropriate volume of solution and then empty its contents into the tube, thus replacing the original tube contents), or through a constant drip. The rapid turnover method is certainly simpler, however when the media/food is introduced at once, the stability of the thermocline is lost. And while the constant drip method is certainly more labor intensive (it requires the set up of a peristaltic pump and tubing), I prefer this method since it allows for a more constant inter-tube environment.

In order to maintain the appropriate quantity and quality of food in the tubes, each day I collected fresh algae output from chemostats, measured the color spectrophotometrically, and then added the appropriate amount of each food type to media in an inflow vessel (in my case, a 4 liter beaker). Each day extra food/media in the inflow vessel was discarded, the inflow vessel was washed thoroughly in hot water to remove bacteria, and fresh media and food were replaced.

Fish kairomone may be added to the tubes. In order to add this mysterious group of chemicals, incubate 2 goldfish (approximately 5 cm each) in approximately 4 liters of water for about 2 hours. This “fish water” can then be dripped into the tubes. Do note that if you are concerned with the stoichiometry of the inflow media, fish should be kept in a separate inflow vessel filled with N and P free media, and should be dripped in separately from the media + food. Fish obviously excrete high levels of N and this will change the nutrient ratio of media used. In addition, any materials used in the experiment (i.e. beakers, pipettes) should be labeled for “fish” or “non-fish” tubes, so as to prevent the accidental introduction of any kairomone into kairomone-free tubes.

Since bacteria degrades kairomone, it is imperative to use clean tubing at the beginning of the experiment. It may be helpful as well to use a small diameter of tubing so as to keep media moving through the tubing at a relatively rapid pace.

Conclusion

Before my trip to Poland, I was unable to maintain a population of successfully migrating *Daphnia.* Upon returning from my trip, I implemented many changes all at once (to make my plankton organ as similar to the Polish plankton organ as possible.) Therefore, I am unable to determine specifically which of the changes I implemented were essential, and which were less important. While understanding specifically which factors/changes I made enhanced or inhibited migration is undoubtedly a very interesting and pertinent question that should be examined in the future; I chose not to pursue these answers. In order to answer my research question and to better understand the role of nutrients in vertical migration, I did not want to disrupt the functional set-up.

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Table 1. Light availability at various locationations in the plankton organ. Readings were taken at 45 degrees to the left, directly up (90 degrees), 45 degrees to the right, 45 degrees facing the front of the plankton organ, and 45 degrees facing the rear of the plankton organ. All light measurements are recorded in μE/m^2/sec.

Location

1	4	7	10
2	5	8	11
3	6	9	12

location 1		location 4		location 7		location 10	
left	0.22	left	0.48	left	1.44	left	3.05
center	6.57	center	13.03	center	13	center	3.93
right	3.63	right	1.76	right	0.29	right	0.18
front	0.27	front	0.16	front	0.13	front	0.67
back	0.2	back	0.68	back	0.18	back	0.12
location 2		location 5		location 8		location 11	
left	0.92	left	0.61	left	1.82	left	3.25
center	5.62	center	9.56	center	10.9	center	5.67
right	1.04	right	0.71	right	0.18	right	0.32
front	0.04	front	0.21	front	0.28	front	0.08
back	0.28	back	0.21	back	0.33	back	0.11
location 3		location 6		location 9		location 12	
left	0.26	left	1.05	left	1.93	left	2.85
center	4.69	center	6.73	center	7	center	4.15
right	2.58	right	0.98	right	0.45	right	2.09
front	0.28	front	0.58	front	0.34	front	0.15
back	0.25	back	2.34	back	1.48	back	0.46

Appendix B

Algal distribution within the plankton organ

Distribution of algal cells two and one-half hours, four and one-half hours, and ten hours after continuous introduction of algae to the top of three plankton organ tubes. Samples were taken by pipette from each of five layers within each tube, and the samples were counted in duplicate using a cell counter. (Data collected June 7, 2001)

		Tube 1	Tube 2	Tube 3
Hour 2.5	Layer 1	140,200	52,170	64,670
	Layer 2	99,080	86,920	74,000
	Layer 3	76,830	105,500	71,500
	Layer 4	83,250	121,000	69,330
	Layer 5	68,670	72,920	66,330
Hour 4.5	Layer 1	213,100	Sample lost	438,500
	Layer 2	164,800	141,200	284,100
	Layer 3	193,000	118,900	302,500
	Layer 4	140,600	155,000	228,800
	Layer 5	158,200	93,330	188,200
Hour 10	Layer 1	1,669,000	1,567,000	2,461,000
	Layer 2	2,550,000	855,800	2,205,000
	Layer 3	2,205,000	750,800	2,363,000
	Layer 4	2,289,000	895,000	2,392,000
	Layer 5	2,290,000	1,187,000	1,528,000

Appendix C

Lake-specific physical, chemical, and biological data

Table 1. Lake name, lake code, Minnesota Department of Natural Resources (MN DNR) lake identification number (for Minnesota lakes only), county, sampling region, sample date, sample time, maximum depth, and secchi depth of each lake. The first four lakes listed are lakes with low C:P in the lower layer and high C:P in the upper layer. The remaining lakes have low C:P throughout the water column and are arranged alphabetically by region.

Lake Name	Lake Code	MN DNR Lake ID	County	Region	Sample date	Sample time (am)	Zmax (m)	Secchi depth (m)
16 Lake	16L		Allegan	MI	6/15/2001	8:05	25	4.2
Derby	DER		Montcalm	MI	6/28/2001	7:35	29	3.6
Green	GRN		Allegan	MI	6/17/2001	8:37	16	2.0
Lee	LEE		Calhoun	MI	6/29/2001	8:15	14	2.3
Okoboji	OKO		Dickinson	IA	5/25/2000	11:30	40	7.3
Barton	BAR		Kalamazoo	MI	6/19/2001		11	2.1
Crispell	CRI		Jackson	MI	6/20/2001	8:40	5	3.3
Goguac	GOG		Calhoun	MI	6/21/2001	8:40	19	2.4
Long (LNG)	LNG		Kalamazoo	MI	6/26/2001	7:40	16	5.1
Miner	MIN		Allegan	MI	6/14/2001	11:15	26	2.0
Bear Island	BIS	69011500	St. Louis	NE MN	6/24/2000	8:25	18	2.0
Bearhead	BRH	69025400	St. Louis	NE MN	6/22/2000	8:15	12	3.5
Burntside	BUR	69011800	St. Louis	NE MN	6/28/2000	7:43	31	4.5
Eagle's Nest 2	EN2	69028502	St. Louis	NE MN	6/8/2000	7:10	10	6.5
Farm	FAR	38077900	Lake	NE MN	6/7/2000	11:15	11	2.0
Fenske	FEN	69008500	St. Louis	NE MN	6/9/2000	8:30	11	2.9
Hogback	HOG	38005700	Lake	NE MN	6/21/2000	8:30	12	3.5
Moose	MOS	16004300	Cook	NE MN	6/19/2000	8:45	19	3.8
Snowbank	SNO	38052900	Lake	NE MN	6/15/2000	8:45	24	5.3
21 Lake	21L	29013000	Hubard	NW MN	7/17/2000	8:40	16	4.0
2 Inlets	2IN	3001700	Becker	NW MN	7/10/2000	9:30	12	3.0
Beltrami	BEL	4013500	Beltrami	NW MN	7/28/2000	6:20	14	4.6
Elk	ELK	15001000	Clearwater	NW MN	7/27/2001	8:25	30	3.4
Itasca	ITA	15001600	Clearwater	NW MN	7/19/2000	8:15	11	1.3
Long (LON)	LON	15005700	Clearwater	NW MN	7/12/2000	7:30	21	4.5
Many Point	MAN	3015800	Becker	NW MN	7/15/2000	9:40	20	2.1
Newman	NEW	29023700	Hubbard	NW MN	7/23/2000	8:15	19	
Plantagenet	PLA	29015600	Hubbard	NW MN	7/24/2000	8:35	19	2.2
Little Sand	SAN	29015000	Hubbard	NW MN	7/20/2000	8:25	23	6.1
Upper Bottle	UPB	29014800	Hubbard	NW MN	7/14/2000	8:15	11	4.6
Christmas	XMS	27013700	Hennepin	TC	8/12/2000	7:40	25	3.0
Harriet	HAR	27001600	Hennepin	TC	8/14/2000	7:10	19	2.0
Josephine	JOS	62005700	Ramsey	TC	8/7/2000	7:00	12	1.5
Minnetonka	MTK	27013300	Hennepin	TC	8/15/2000	7:30	23	2.8
White Bear	WBL	82016700	Washington	TC	8/9/2000	8:55	24	3.0

Table 2A. Layer thickness (in meters) of each stratum (epilimnion, metalimnion, and hypolimnion) of each of the study lakes. The upper temperature column represents the average temperature weighted by epilimentic and metalimnetic layer thickness (see Chapter 4, equation 1 for calculation). The lower temperature column is the same as the hypo column, as no conversions were necessary for that data. The first four lakes listed are lakes with low C:P in the lower layer and high C:P in the upper layer. The remaining lakes have low C:P throughout the water column and are sorted by upper layer seston C:P (See Table 2B).

Lake Code	Layer Thickess (m)			Temperature (°C)	
	epi	meta	hypo	Upper	Lower
DER	3.00	10.00	16.00	18.50	4.67
LEE	2.00	7.00	5.00	19.77	9.72
16L	1.00	10.00	14.00	15.03	4.86
GRN	3.00	6.00	7.00	18.72	7.85
FEN	2.50	4.50	4.00	14.28	5.65
JOS	5.00	4.00	3.00	20.16	8.65
SNO	9.00	4.00	11.00	14.06	7.47
BRH	8.00	2.00	2.00	15.72	9.20
HOG	4.00	4.00	4.00	13.59	5.19
HAR	5.00	5.00	9.00	20.08	3.60
CRI	2.00	1.00	2.00	22.63	16.33
LNG	3.00	6.00	7.00	13.89	0.00
LON	7.00	6.00	8.00	17.01	5.91
ELK	6.00	5.00	19.00	16.30	5.33
BAR	2.00	2.00	7.00	20.88	11.76
MIN	1.00	8.00	17.00	17.27	7.00
NEW	4.00	5.00	10.00	18.08	5.33
SAN	6.00	4.00	13.00	17.70	6.59
XMS	6.00	4.00	15.00	21.41	6.75
GOG	3.00	10.00	6.00	19.64	6.66
BUR	8.00	7.00	16.00	14.53	3.89
21L	4.00	5.00	7.00	19.39	5.84
MTK	7.00	9.00	7.00	21.08	8.91
MAN	5.00	9.00	6.00	18.94	9.39
WBL	9.00	8.00	7.00	20.44	8.80
ITA	5.00	4.00	2.00	15.49	13.79
BIS	4.00	9.00	5.00	14.00	9.95
MOS	7.00	4.00	8.00	14.78	8.85
BEL	4.00	6.00	4.00	19.62	8.85
UPB	3.00	6.00	2.00	19.71	8.96
2IN	2.00	8.00	2.00	17.80	8.75
EN2	4.00	4.00	2.00	16.93	10.60
OKO	16.00	12.00	12.00	13.71	8.75
FAR	4.00	4.00	3.00	15.90	13.70
PLA	7.00	5.00	7.00	18.60	10.77

Table 2B. Seston C:P of each stratum (epilimnion, metalimnion, and hypolimnion) of each of the study lakes. The upper seston C:P column represents the average C:P weighted by epilimentic and metalimnetic layer thickness (see Chapter 4, equation 1 for calculation). The lower C:P column is the same as the hypo column, as no conversions were necessary for that data. The first four lakes listed are lakes with low C:P in the lower layer and high C:P in the upper layer. The remaining lakes have low C:P throughout the water column and are sorted by upper layer seston C:P.

Lake Code	Seston C:P			Seston C:P	
	epi	meta	hypo	Upper	Lower
DER	338.94	640.37	184.50	570.81	184.50
LEE	419.34	549.92	132.60	520.90	132.60
16L	507.32	497.78	179.57	498.65	179.57
GRN	300.24	331.76	150.10	321.25	150.10
FEN	389.39	224.50	225.56	283.39	225.56
JOS	376.69	146.48	14.32	274.38	14.32
SNO	305.07	191.67	134.18	270.18	134.18
BRH	294.35	93.98	107.30	254.28	107.30
HOG	308.24	182.21	238.74	245.22	238.74
HAR	287.17	187.21	100.54	237.19	100.54
CRI	233.54	236.25	181.03	234.45	181.03
LNG	275.56	211.89	148.29	233.12	148.29
LON	226.71	236.65	90.49	231.29	90.49
ELK	264.21	166.43	50.62	219.77	50.62
BAR	191.54	240.50	127.89	216.02	127.89
MIN	257.31	208.69	204.03	214.09	204.03
NEW	70.11	319.75	174.57	208.80	174.57
SAN	216.68	192.52	67.09	207.02	67.09
XMS	218.91	158.76	46.72	194.85	46.72
GOG	261.84	169.52	189.74	190.83	189.74
BUR	192.01	164.20	125.79	179.03	125.79
21L	163.08	177.71	100.66	171.21	100.66
MTK	198.40	141.85	116.45	166.59	116.45
MAN	176.81	140.20	39.26	153.28	39.26
WBL	242.72	48.93	27.16	151.53	27.16
ITA	156.29	143.71	57.48	150.70	57.48
BIS	179.99	113.07	77.02	133.66	77.02
MOS	135.92	124.90	30.42	131.91	30.42
BEL	149.80	119.64	69.96	131.70	69.96
UPB	134.89	114.83	95.05	121.51	95.05
2IN	144.31	91.60	24.32	102.14	24.32
EN2	95.50	103.07	76.10	99.28	76.10
OKO	90.84	59.51	60.85	77.41	60.85
FAR	74.69	65.33	70.92	70.01	70.92
PLA	77.92	35.82	6.06	60.38	6.06

Table 3. Lake, Minnesota Department of Natural Resources (MN DNR) lake identification number (for Minnesota lakes only), and abundance per gill net of planktivores in each lake. Year sampled represents the year gill net sampling was performed. NA represents no fish data available. UNK represents unknown number of net samples as the number of net samples taken was not provided by the MN DNR. The first four lakes listed are lakes with low C:P in the lower layer and high C:P in the upper layer. The remaining lakes have low C:P throughout the water column and are arranged by total planktivore abundance.

Lake Code	MN DNR Lake ID	Year sampled	Number of nets taken	Number of planktivores/gill net				
				black crappie	white crappie	bluegills	yellow perch	Total
GRN		2001	1	8.00	0.00	11.00	22.00	41.00
DER		2001	8	0.00	0.00	0.75	10.13	10.88
LEE		2001	2	3.00	0.00	3.00	0.00	6.00
16L		NA	NA					
ITA	15001600	2000	12	0.42	0.00	2.33	144.50	147.25
LON	15005700	2001	6	0.00	0.00	0.17	102.83	103.00
HAR	27001600	2000	6	19.00	0.00	29.00	46.67	94.67
PLA	29015600	1999	15	0.00	0.00	0.00	50.47	50.47
2IN	3001700	2001	9	2.33	0.00	1.89	35.56	39.78
MTK	27013300		24	3.79	0.00	24.33	4.88	33.00
BEL	4013500	2000	UNK	1.10	0.00	4.40	27.10	32.60
WBL	82016700	1999	14	2.50	0.00	20.93	6.79	30.21
ELK	15001000	2000	UNK	0.00	0.00	0.80	28.80	29.60
UPB	29014800	2003	6	2.33	0.00	1.33	17.33	21.00
EN2	69028502	2000	6	0.00	0.00	5.67	14.67	20.33
BAR		2001	4	3.50	0.00	0.50	9.50	13.50
BRH	69025400	1999	12	0.33	0.00	2.17	10.25	12.75
GOG		2001	2	1.50	0.00	11.00	0.00	12.50
JOS	62005700	2001	4	2.50	0.00	9.00	0.00	11.50
MAN	3015800	1998	12	0.08	0.00	0.42	10.67	11.17
21L	29013000	2003	2	0.00	0.00	6.50	2.00	8.50
SAN	29015000	2002	9	0.11	0.00	1.00	5.00	6.11
XMS	27013700	2001	6	0.00	0.00	4.33	0.50	4.83
LNG		2001	8	1.38	0.00	1.00	1.25	3.63
HOG	38005700	1999	2	0.00	0.00	0.00	3.00	3.00
SNO	38052900	2000	11	0.00	0.00	0.00	2.73	2.73
FAR	38077900	1999	7	0.14	0.00	0.57	1.86	2.57
BIS	69011500	1998	15	0.33	0.00	0.07	1.47	1.87
MOS	16004300	1998	6	0.00	0.00	1.30	0.30	1.60
FEN	69008500	1997	6	0.00	0.00	0.17	0.33	0.50
BUR	69011800	1999	12	0.25	0.00	0.08	0.00	0.33
NEW	29023700	1998	2	0.00	0.00	0.00	0.00	0.00
OKO		2001	24	0.00	0.00	0.00	0.00	0.00
CRI		NA	NA					
MIN		NA	NA					

Table 4A. Number of total *D. retrocurva* per liter per stratum (epilimnion, metalimnion, and hypolimnion) and per layer (upper and lower), and the percent *D. retrocurva* in each layer. The upper data column represents the average total *D. retrocurva* per liter weighted by epilimentic and metalimnetic layer thickness (see Chapter 4, equation 1 for calculation). The lower data column is the same as the hypo column, as no conversions were necessary for that data. Percent upper and percent lower columns represent the percent (out of 100%) of the total *D. retrocurva* located in the upper layer and lower layers. NA in the percent upper and percent lower columns represents lakes with no *D. retrocurva*. The first four lakes listed are lakes with low C:P in the lower layer and high C:P in the upper layer. The remaining lakes have low C:P throughout the water column and are arranged alphabetically.

Lake Code	*D. retrocurva*						
	epi	meta	hypo	upper	lower	% upper	% lower
16L	0.00	0.00	0.00	0.00	0.00	NA	NA
DER	0.00	0.00	0.00	0.00	0.00	NA	NA
GRN	11.48	19.97	12.93	17.14	12.93	56.99	43.01
LEE	0.00	0.00	0.00	0.00	0.00	NA	NA
21L	0.00	0.00	0.00	0.00	0.00	NA	NA
2IN	0.00	0.00	0.00	0.00	0.00	NA	NA
BAR	0.00	0.00	0.00	0.00	0.00	NA	NA
BEL	0.00	0.00	0.00	0.00	0.00	NA	NA
BIS	0.00	0.00	0.00	0.00	0.00	NA	NA
BRH	0.00	0.00	0.00	0.00	0.00	NA	NA
BUR	0.00	0.00	0.00	0.00	0.00	NA	NA
CRI	0.00	0.00	0.00	0.00	0.00	NA	NA
ELK	0.80	0.00	0.87	0.44	0.87	33.49	66.51
EN2	0.00	0.00	0.00	0.00	0.00	NA	NA
FAR	0.00	0.00	0.00	0.00	0.00	NA	NA
FEN	0.00	0.00	0.00	0.00	0.00	NA	NA
GOG	11.07	10.30	2.75	10.48	2.75	79.21	20.79
HAR	1.05	0.90	0.00	0.98	0.00	100.00	0.00
HOG	0.00	0.00	0.00	0.00	0.00	NA	NA
ITA	0.00	0.00	0.00	0.00	0.00	NA	NA
JOS	0.97	0.83	2.90	0.91	2.90	23.83	76.17
LNG	0.00	0.00	0.00	0.00	0.00	NA	NA
LON	0.00	0.00	0.00	0.00	0.00	NA	NA
MAN	2.72	14.95	3.40	10.58	3.40	75.68	24.32
MIN	1.93	0.00	0.00	0.21	0.00	100.00	0.00
MOS	1.83	1.78	0.00	1.81	0.00	100.00	0.00
MTK	20.03	3.52	9.73	10.74	9.73	52.46	47.54
NEW	0.00	0.00	0.00	0.00	0.00	NA	NA
OKO	0.00	0.00	0.00	0.00	0.00	NA	NA
PLA	0.00	0.00	0.00	0.00	0.00	NA	NA
SAN	0.00	0.00	0.00	0.00	0.00	NA	NA
SNO	0.00	0.00	0.00	0.00	0.00	NA	NA
UPB	0.00	0.00	0.00	0.00	0.00	NA	NA
WBL	21.70	6.73	4.20	14.66	4.20	77.73	22.27
XMS	7.33	2.58	0.92	5.43	0.92	85.56	14.44

Table 4B. Number of total *D. mendotae* per liter per stratum (epilimnion, metalimnion, and hypolimnion) and per layer (upper and lower), and the percent *D. mendotae* in each layer. The upper data column represents the average total *D. mendotae* per liter weighted by epilimentic and metalimnetic layer thickness (see Chapter 4, equation 1 for calculation). The lower data column is the same as the hypo column, as no conversions were necessary for that data. Percent upper and percent lower columns represent the percent (out of 100%) of the total *D. mendotae* located in the upper layer and lower layers. NA in the percent upper and percent lower columns represents lakes with no *D. mendotae* . The first four lakes listed are lakes with low C:P in the lower layer and high C:P in the upper layer. The remaining lakes have low C:P throughout the water column and are arranged alphabetically.

Lake Code	*D. mendotae*						
	epi	meta	hypo	upper	lower	% upper	% lower
16L	0.00	0.00	0.00	0.00	0.00	NA	NA
DER	0.00	0.00	0.00	0.00	0.00	NA	NA
GRN	0.95	2.23	1.35	1.80	1.35	57.14	42.86
LEE	0.00	0.00	1.53	0.00	1.53	0.00	100.00
21L	0.78	0.78	1.85	0.78	1.85	29.62	70.38
2IN	26.18	18.68	4.53	20.18	4.53	81.66	18.34
BAR	0.00	0.00	0.00	0.00	0.00	NA	NA
BEL	0.80	2.95	0.00	2.09	0.00	100.00	0.00
BIS	4.93	3.00	4.13	3.59	4.13	46.52	53.48
BRH	13.20	12.00	14.00	12.96	14.00	48.07	51.93
BUR	2.41	3.55	1.35	2.94	1.35	68.54	31.46
CRI	0.00	0.00	0.00	0.00	0.00	NA	NA
ELK	0.00	0.00	0.00	0.00	0.00	NA	NA
EN2	20.80	14.75	11.93	17.78	11.93	59.83	40.17
FAR	4.38	3.37	2.00	3.87	2.00	65.93	34.07
FEN	0.97	7.03	0.75	4.86	0.75	86.63	13.37
GOG	0.00	0.00	0.00	0.00	0.00	NA	NA
HAR	1.93	0.90	0.90	1.41	0.90	61.08	38.92
HOG	13.75	2.35	6.00	8.05	6.00	57.30	42.70
ITA	0.00	8.56	0.00	3.80	0.00	100.00	0.00
JOS	0.00	0.00	0.00	0.00	0.00	NA	NA
LNG	0.00	0.00	0.00	0.00	0.00	NA	NA
LON	0.00	0.00	0.00	0.00	0.00	NA	NA
MAN	0.00	0.00	0.00	0.00	0.00	NA	NA
MIN	39.93	24.70	5.07	26.39	5.07	83.89	16.11
MOS	0.00	1.08	4.08	0.39	4.08	8.79	91.21
MTK	0.00	1.73	0.00	0.97	0.00	100.00	0.00
NEW	4.60	8.08	3.37	6.54	3.37	66.00	34.00
OKO	0.00	0.00	0.00	0.00	0.00	NA	NA
PLA	0.92	1.48	0.00	1.15	0.00	100.00	0.00
SAN	0.00	0.00	0.00	0.00	0.00	NA	NA
SNO	2.72	4.60	0.00	3.30	0.00	100.00	0.00
UPB	0.00	0.00	5.00	0.00	5.00	0.00	100.00
WBL	0.00	0.00	0.00	0.00	0.00	NA	NA
XMS	1.70	1.68	0.83	1.69	0.83	67.02	32.98

Table 4C. Number of total *D. pulicaria* per liter per stratum (epilimnion, metalimnion, and hypolimnion) and per layer (upper and lower), and the percent D. pulicaria in each layer. The upper data column represents the average total *D. pulicaria* per liter weighted by epilimentic and metalimnetic layer thickness (see Chapter 4, equation 1 for calculation). The lower data column is the same as the hypo column, as no conversions were necessary for that data. Percent upper and percent lower columns represent the percent (out of 100%) of the total *D. pulicaria* located in the upper layer and lower layers. NA in the percent upper and percent lower columns represents lakes with no *D. pulicaria* . The first four lakes listed are lakes with low C:P in the lower layer and high C:P in the upper layer. The remaining lakes have low C:P throughout the water column and are arranged alphabetically.

Lake Code	*D. pulicaria*						
	epi	meta	hypo	upper	lower	% upper	% lower
16L	0.00	2.48	2.75	2.26	2.75	45.08	54.92
DER	0.00	0.00	4.02	0.00	4.02	0.00	100.00
GRN	0.95	0.00	0.00	0.32	0.00	100.00	0.00
LEE	7.50	0.98	8.13	2.43	8.13	23.01	76.99
21L	0.00	0.00	0.00	0.00	0.00	NA	NA
2IN	0.00	0.00	0.00	0.00	0.00	NA	NA
BAR	0.00	0.00	0.00	0.00	0.00	NA	NA
BEL	2.30	0.00	0.00	0.92	0.00	100.00	0.00
BIS	0.00	0.00	0.70	0.00	0.70	0.00	100.00
BRH	0.00	6.45	0.00	1.29	0.00	100.00	0.00
BUR	0.00	0.00	0.00	0.00	0.00	NA	NA
CRI	0.00	6.70	3.15	2.23	3.15	41.49	58.51
ELK	0.00	0.00	0.00	0.00	0.00	NA	NA
EN2	0.00	0.00	0.00	0.00	0.00	NA	NA
FAR	0.00	3.87	4.00	1.93	4.00	32.58	67.42
FEN	3.70	2.60	4.33	2.99	4.33	40.90	59.10
GOG	2.83	3.18	0.00	3.09	0.00	100.00	0.00
HAR	0.00	0.00	0.00	0.00	0.00	NA	NA
HOG	0.00	0.00	1.65	0.00	1.65	0.00	100.00
ITA	0.00	0.00	0.75	0.00	0.75	0.00	100.00
JOS	0.00	0.00	0.00	0.00	0.00	NA	NA
LNG	0.00	0.00	5.40	0.00	5.40	0.00	100.00
LON	0.00	0.70	2.97	0.32	2.97	9.82	90.18
MAN	0.00	0.00	0.00	0.00	0.00	NA	NA
MIN	0.00	2.68	13.62	2.39	13.62	14.91	85.09
MOS	0.00	0.00	0.00	0.00	0.00	NA	NA
MTK	3.42	0.00	0.00	1.49	0.00	100.00	0.00
NEW	0.00	0.00	0.00	0.00	0.00	NA	NA
OKO	12.76	7.50	7.12	10.50	7.12	59.61	40.39
PLA	2.87	0.00	0.00	1.67	0.00	100.00	0.00
SAN	0.00	0.00	0.00	0.00	0.00	NA	NA
SNO	0.00	0.87	0.90	0.27	0.90	22.86	77.14
UPB	0.00	0.00	0.00	0.00	0.00	NA	NA
WBL	0.83	0.00	0.00	0.44	0.00	100.00	0.00
XMS	0.77	2.30	2.60	1.38	2.60	34.67	65.33

Table 4D. Number of total *D. ambigua* per liter per stratum (epilimnion, metalimnion, and hypolimnion) and per layer (upper and lower), and the percent *D. ambigua* in each layer. The upper data column represents the average total *D. ambigua* per liter weighted by epilimentic and metalimnetic layer thickness (see Chapter 4, equation 1 for calculation). The lower data column is the same as the hypo column, as no conversions were necessary for that data. Percent upper and percent lower columns represent the percent (out of 100%) of the total *D. ambigua* located in the upper layer and lower layers. NA in the percent upper and percent lower columns represents lakes with no *D. ambigua*. The first four lakes listed are lakes with low C:P in the lower layer and high C:P in the upper layer. The remaining lakes have low C:P throughout the water column and are arranged alphabetically.

Lake Code	*D. ambigua*						
	epi	meta	hypo	upper	lower	% upper	% lower
16L	0.00	0.00	0.00	0.00	0.00	NA	NA
DER	0.00	0.00	0.00	0.00	0.00	NA	NA
GRN	0.00	0.00	0.00	0.00	0.00	NA	NA
LEE	0.00	0.00	0.00	0.00	0.00	NA	NA
21L	0.00	0.00	0.00	0.00	0.00	NA	NA
2IN	0.00	0.00	0.00	0.00	0.00	NA	NA
BAR	0.00	0.00	0.00	0.00	0.00	NA	NA
BEL	0.00	0.00	0.00	0.00	0.00	NA	NA
BIS	0.00	0.00	0.00	0.00	0.00	NA	NA
BRH	0.00	0.00	0.00	0.00	0.00	NA	NA
BUR	0.00	0.00	0.00	0.00	0.00	NA	NA
CRI	0.00	0.00	0.00	0.00	0.00	NA	NA
ELK	0.00	0.00	0.00	0.00	0.00	NA	NA
EN2	0.00	0.00	0.00	0.00	0.00	NA	NA
FAR	0.00	0.00	0.00	0.00	0.00	NA	NA
FEN	0.00	0.00	0.00	0.00	0.00	NA	NA
GOG	0.00	0.00	0.00	0.00	0.00	NA	NA
HAR	0.00	0.00	0.00	0.00	0.00	NA	NA
HOG	0.00	0.00	0.00	0.00	0.00	NA	NA
ITA	0.00	0.00	0.00	0.00	0.00	NA	NA
JOS	0.00	0.00	0.00	0.00	0.00	NA	NA
LNG	0.00	0.00	0.00	0.00	0.00	NA	NA
LON	0.00	0.00	0.00	0.00	0.00	NA	NA
MAN	0.00	0.00	0.00	0.00	0.00	NA	NA
MIN	0.00	0.00	0.00	0.00	0.00	NA	NA
MOS	0.00	0.00	0.00	0.00	0.00	NA	NA
MTK	0.00	0.00	0.00	0.00	0.00	NA	NA
NEW	0.00	0.00	0.00	0.00	0.00	NA	NA
OKO	0.00	7.50	0.00	3.21	0.00	100.00	0.00
PLA	0.00	0.00	0.00	0.00	0.00	NA	NA
SAN	0.60	6.83	4.87	3.09	4.87	38.86	61.14
SNO	0.00	0.00	0.00	0.00	0.00	NA	NA
UPB	0.00	0.00	0.00	0.00	0.00	NA	NA
WBL	0.00	0.00	0.00	0.00	0.00	NA	NA
XMS	0.00	0.00	0.00	0.00	0.00	NA	NA

Table 4E Number of total *Daphnia* spp. per liter per stratum (epilimnion, metalimnion, and hypolimnion) and per layer (upper and lower), and the percent *Daphnia* in each layer. The upper data column represents the average total *Daphnia* spp. per liter weighted by epilimentic and metalimnetic layer thickness (see Chapter 4, equation 1 for calculation). The lower data column is the same as the hypo column, as no conversions were necessary for that data. Percent upper and percent lower columns represent the percent (out of 100%) of the total *Daphnia* spp. located in the upper layer and lower layers. NA in the percent upper and percent lower columns represents lakes with no *Daphnia* spp. The first four lakes listed are lakes with low C:P in the lower layer and high C:P in the upper layer. The remaining lakes have low C:P throughout the water column and are arranged alphabetically.

Lake Code	Upper C:P	Total *Daphnia* spp.						
		epi	meta	hypo	upper	lower	% upper	% lower
16L	high	0.00	2.48	2.75	2.26	2.75	45.08	54.92
DER	high	0.00	0.00	4.02	0.00	4.02	0.00	100.00
GRN	high	13.38	22.19	14.28	19.26	14.28	57.41	42.59
LEE	high	7.50	0.98	9.67	2.43	9.67	20.10	79.90
21L	low	0.78	0.78	1.85	0.78	1.85	29.62	70.38
2IN	low	26.18	18.68	4.53	20.18	4.53	81.66	18.34
BAR	low	0.00	0.00	0.00	0.00	0.00	NA	NA
BEL	low	3.10	2.95	0.00	3.01	0.00	100.00	0.00
BIS	low	4.93	3.00	4.83	3.59	4.83	42.65	57.35
BRH	low	13.20	18.45	14.00	14.25	14.00	50.44	49.56
BUR	low	2.41	3.55	1.35	2.94	1.35	68.54	31.46
CRI	low	0.00	6.70	3.15	2.23	3.15	41.49	58.51
ELK	low	0.80	0.00	0.87	0.44	0.87	33.49	66.51
EN2	low	20.80	14.75	11.93	17.78	11.93	59.83	40.17
FAR	low	4.38	7.23	6.00	5.80	6.00	49.17	50.83
FEN	low	4.67	9.63	5.08	7.85	5.08	60.75	39.25
GOG	low	13.89	13.48	2.75	13.57	2.75	83.15	16.85
HAR	low	2.98	1.80	0.90	2.39	0.90	72.62	27.38
HOG	low	13.75	2.35	7.65	8.05	7.65	51.27	48.73
ITA	low	0.00	8.56	0.75	3.80	0.75	83.53	16.47
JOS	low	0.97	0.83	2.90	0.91	2.90	23.83	76.17
LNG	low	0.00	0.00	5.40	0.00	5.40	0.00	100.00
LON	low	0.00	0.70	2.97	0.32	2.97	9.82	90.18
MAN	low	2.72	14.95	3.40	10.58	3.40	75.68	24.32
MIN	low	41.87	27.38	18.68	28.99	18.68	60.81	39.19
MOS	low	1.83	2.86	4.08	2.21	4.08	35.13	64.87
MTK	low	23.45	5.24	9.73	13.21	9.73	57.57	42.43
NEW	low	4.60	8.08	3.37	6.54	3.37	66.00	34.00
OKO	low	12.76	15.00	7.12	13.72	7.12	65.84	34.16
PLA	low	3.78	1.48	0.00	2.83	0.00	100.00	0.00
SAN	low	0.60	6.83	4.87	3.09	4.87	38.86	61.14
SNO	low	2.72	5.47	0.90	3.56	0.90	79.83	20.17
UPB	low	0.00	0.00	5.00	0.00	5.00	0.00	100.00
WBL	low	22.53	6.73	4.20	15.10	4.20	78.24	21.76
XMS	low	9.80	6.57	4.35	8.51	4.35	66.17	33.83

Table 5. Lake name, lake identification code, sample date, enumeration date, total sample volume, and raw data counts for each of three one ml samples collected from sampled lakes. In general, each stratum (epi, meta, or hypo) was divided into two sections (1 and 2). Each section within each stratum was sampled in duplicate (A and B). Four species of *Daphnia* were identified in the samples. Letters A, B, and C under each species header represent the three samples enumerated.

Lake Name	Lake Identification Code	Sample Date	Count Date	Total Volume	*D. mendotae*			*D. pulicaria*			*D. ambigua*			*D. retrocurva*		
					A	B	C	A	B	C	A	B	C	A	B	C
16 Lake	16L EPI 1A	6/15/2001	1/3/2004	51												
16 Lake	16L EPI 1B	6/15/2001	1/3/2004	43												
16 Lake	16L META 1A	6/15/2001	1/3/2004	42												
16 Lake	16L META 1B	6/15/2001	1/3/2004	48				1								
16 Lake	16L META 2A	6/15/2001	1/3/2004	53				1								
16 Lake	16L META 2B	6/15/2001	1/3/2004	48				1								
16 Lake	16L HYPO 1A	6/15/2001	1/3/2004	56				1	1	1						
16 Lake	16L HYPO 1B	6/15/2001	1/3/2004	61												
16 Lake	16L HYPO 2A	6/15/2001	1/3/2004	47												
16 Lake	16L HYPO 2B	6/15/2001	1/3/2004	46												
Derby	DER EPI 1A	6/28/2001	1/7/2004	47												
Derby	DER EPI 1B	6/28/2001	1/7/2004	44												
Derby	DER EPI 2A	6/28/2001	1/7/2004	45												
Derby	DER META 1A	6/28/2001	1/7/2004	43												
Derby	DER META 1B	6/28/2001	1/7/2004	35												
Derby	DER META 2A	6/28/2001	1/7/2004	43												
Derby	DER META 2B	6/28/2001	1/7/2004	39												
Derby	DER HYPO 1A	6/28/2001	1/7/2004	48				1								
Derby	DER HYPO 1B	6/28/2001	1/7/2004	45					2	1						
Derby	DER HYPO 2A	6/28/2001	1/7/2004	58				1								
Derby	DER HYPO 2B	6/28/2001	1/7/2004	48												

Table 5 continued.

Lake Name	Lake Identification Code	Sample Date	Count Date	Total Volume	D. mendotae			D. pulicaria			D. ambigua			D. retrocurva		
					A	B	C	A	B	C	A	B	C	A	B	C
Green	GRN EPI 1A	6/17/2001	1/3/2004	57	1			1								
Green	GRN EPI 1B	6/17/2001	1/3/2004	38											2	
Green	GRN EPI 2A	6/17/2001	1/3/2004	43										1	2	
Green	GRN EPI 2B	6/17/2001	1/3/2004	44										3	4	4
Green	GRN META 1A	6/17/2001	1/3/2004	45										1	1	2
Green	GRN META 1B	6/17/2001	1/3/2004	46	1		1							2	6	3
Green	GRN META 2A	6/17/2001	1/3/2004	42			1							2	1	1
Green	GRN META 2B	6/17/2001	1/3/2004	43										2	2	4
Green	GRN HYPO 1A	6/17/2001	1/3/2004	55										1	4	
Green	GRN HYPO 1B	6/17/2001	1/3/2004	49											1	6
Green	GRN HYPO 2A	6/17/2001	1/3/2004	38			1								1	2
Green	GRN HYPO 2B	6/17/2001	1/3/2004	44			1									1
Lee	LEE EPI 1A	6/29/2001	1/7/2004	55				3		1						
Lee	LEE EPI 1B	6/29/2001	1/7/2004	47												
Lee	LEE META 1A	6/29/2001	1/7/2004	60												
Lee	LEE META 1B	6/29/2001	1/7/2004	60												
Lee	LEE META 2A	6/29/2001	1/7/2004	59					1							
Lee	LEE META 2B	6/29/2001	1/7/2004	59												
Lee	LEE HYPO 1A	6/29/2001	1/7/2004	46			1		1							
Lee	LEE HYPO 1B	6/29/2001	1/7/2004	66				2	1							
21 Lake	21L EPI 1A		1/8/2004	56												
21 Lake	21L EPI 1B		1/8/2004	47	1											
21 Lake	21L EPI 2A		1/8/2004	54												
21 Lake	21L EPI 2B		1/8/2004	48												
21 Lake	21L META 1A		1/8/2004	52												
21 Lake	21L META 1B		1/8/2004	42												
21 Lake	21L META 2A		1/8/2004	56												
21 Lake	21L META 2B		1/8/2004	46		1										
21 Lake	21L HYPO 1A		1/8/2004	53												
21 Lake	21L HYPO 1B		1/8/2004	59		1										
21 Lake	21L HYPO 2A		1/8/2004	46												
21 Lake	21L HYPO 2B		1/8/2004	52			1									

Table 5 continued.

Lake Name	Lake Identification Code	Sample Date	Count Date	Total Volume	*D. mendotae*			*D. pulicaria*			*D. ambigua*			*D. retrocurva*		
					A	B	C	A	B	C	A	B	C	A	B	C
2 Inlets	2IN EPI 1A	7/10/2000	12/23/2003	51	2	1	3									
2 Inlets	2IN EPI 1B	7/10/2000	12/23/2003	55		1										
2 Inlets	2IN EPI 2A	7/10/2000	12/23/2003	55	1	7	2									
2 Inlets	2IN EPI 2B	7/10/2000	12/23/2003	60	5	2	4									
2 Inlets	2IN META 1A	7/10/2000	12/23/2003	46	2	4										
2 Inlets	2IN META 1B	7/10/2000	12/23/2003	50	1	1	1									
2 Inlets	2IN META 2A	7/10/2000	12/23/2003	49	2	4	5									
2 Inlets	2IN META 2B	7/10/2000	12/23/2003	52		1	2									
2 Inlets	2IN HYPO 1A	7/10/2000	12/23/2003	45			2									
2 Inlets	2IN HYPO 1B	7/10/2000	12/23/2003	46		1										
Barton	BAR EPI 1A	6/19/2001	1/4/2004	54												
Barton	BAR EPI 1B	6/19/2001	1/4/2004	46												
Barton	BAR EPI 2A	6/19/2001	1/4/2004	44												
Barton	BAR EPI 2B	6/19/2001	1/4/2004	43												
Barton	BAR META 1A	6/19/2001	1/4/2004	50												
Barton	BAR META 1B	6/19/2001	1/4/2004	38												
Barton	BAR META 2A	6/19/2001	1/4/2004	40												
Barton	BAR META 2B	6/19/2001	1/4/2004	50												
Barton	BAR HYPO 1A	6/19/2001	1/4/2004	44												
Barton	BAR HYPO 1B	6/19/2001	1/4/2004	41												
Beltrami	BEL EPI 1A	7/28/2000	12/9/2003	47			1		1							
Beltrami	BEL EPI 1B	7/28/2000	12/9/2003	46				1	1							
Beltrami	BEL EPI 2A	7/28/2000	12/9/2003	48												
Beltrami	BEL EPI 2B	7/28/2000	12/9/2003	48												
Beltrami	BEL META 1A	7/28/2000	12/9/2003	50	1	1	1									
Beltrami	BEL META 1B	7/28/2000	12/9/2003	27		1										
Beltrami	BEL META 2A	7/28/2000	12/9/2003	50												
Beltrami	BEL META 2B	7/28/2000	12/9/2003	46												
Beltrami	BEL HYPO 1A	7/28/2000	12/9/2003	55												
Beltrami	BEL HYPO 1B	7/28/2000	12/9/2003	50												
Beltrami	BEL HYPO 2A	7/28/2000	12/9/2003	56												
Beltrami	BEL HYPO 2B	7/28/2000	12/9/2003	63												

Table 5 continued.

Lake Name	Lake Identification Code	Sample Date	Count Date	Total Volume	*D. mendotae*			*D. pulicaria*			*D. ambigua*			*D. retrocurva*		
					A	B	C	A	B	C	A	B	C	A	B	C
Bear Island	BIS EPI 1A	6/24/2000	12/5/2003	43			2									
Bear Island	BIS EPI 1B	6/24/2000	12/5/2003	41	1	1	2									
Bear Island	BIS EPI 2A	6/24/2000	12/5/2003	46		1										
Bear Island	BIS EPI 2B	6/24/2000	12/5/2003	50												
Bear Island	BIS META 1A	6/24/2000	12/5/2003	51			1									
Bear Island	BIS META 1B	6/24/2000	12/5/2003	40	1											
Bear Island	BIS META 2A	6/24/2000	12/5/2003	47		1										
Bear Island	BIS META 2B	6/24/2000	12/5/2003	44			1									
Bear Island	BIS HYPO 1A	6/24/2000	12/5/2003	48		1										
Bear Island	BIS HYPO 1B	6/24/2000	12/5/2003	41	1					1						
Bear Island	BIS HYPO 2A	6/24/2000	12/5/2003	57	1		1									
Bear Island	BIS HYPO 2B	6/24/2000	12/5/2003	45	1											
Bearhead	BRH EPI 1A	6/22/2000	12/5/2003	42												
Bearhead	BRH EPI 1B	6/22/2000	12/5/2003	44	1	1	2									
Bearhead	BRH EPI 2A	6/22/2000	12/5/2003	45	3	1	2									
Bearhead	BRH EPI 2B	6/22/2000	12/5/2003	57	2	1	3									
Bearhead	BRH META 1A	6/22/2000	12/5/2003	51		2	1	1		2						
Bearhead	BRH META 1B	6/22/2000	12/5/2003	41	1		4	1								
Bearhead	BRH HYPO 1A	6/22/2000	12/5/2003	52				1								
Bearhead	BRH HYPO 1B	6/22/2000	12/5/2003	47			3	1								
Burntside	BUR EPI 1A	6/28/2000	12/9/2003	64			1									
Burntside	BUR EPI 1B	6/28/2000	12/9/2003	48												
Burntside	BUR EPI 2A	6/28/2000	12/9/2003	46												
Burntside	BUR EPI 2B	6/28/2000	12/9/2003	40	2											
Burntside	BUR META 1A	6/28/2000	12/9/2003	41	1		1									
Burntside	BUR META 1B	6/28/2000	12/9/2003	41	1											
Burntside	BUR META 2A	6/28/2000	12/9/2003	41		1										
Burntside	BUR META 2B	6/28/2000	12/9/2003	49			1									
Burntside	BUR HYPO 1A	6/28/2000	12/9/2003	40	1	1										
Burntside	BUR HYPO 1B	6/28/2000	12/9/2003	47												
Burntside	BUR HYPO 2A	6/28/2000	12/9/2003	45												
Burntside	BUR HYPO 2B	6/28/2000	12/9/2003	47												

Table 5 continued.

Lake Name	Lake Identification Code	Sample Date	Count Date	Total Volume (ml)	D. mendotae			D. pulicaria			D. ambigua			D. retrocurva		
					A	B	C	A	B	C	A	B	C	A	B	C
Crispell	CRI EPI 1A	6/20/2001	1/4/2004	42												
Crispell	CRI EPI 1B	6/20/2001	1/4/2004	45												
Crispell	CRI META 1A	6/20/2001	1/4/2004	48				1								
Crispell	CRI META 1B	6/20/2001	1/4/2004	51					2	1						
Crispell	CRI HYPO 1A	6/20/2001	1/4/2004	48						1						
Crispell	CRI HYPO 1B	6/20/2001	1/4/2004	46					1							
Elk	ELK EPI 1A	7/27/2000	12/11/2003	54												
Elk	ELK EPI 1B	7/27/2000	12/11/2003	55												
Elk	ELK EPI 2A	7/27/2000	12/11/2003	54												
Elk	ELK EPI 2B	7/27/2000	12/11/2003	47										1		
Elk	ELK META 1A	7/27/2000	12/11/2003	47												
Elk	ELK META 1B	7/27/2000	12/11/2003	58												
Elk	ELK META 2A	7/27/2000	12/11/2003	50											1	
Elk	ELK META 2B	7/27/2000	12/11/2003	68												
Elk	ELK HYPO 1A	7/27/2000	12/11/2003	60												
Elk	ELK HYPO 1B	7/27/2000	12/11/2003	62												
Elk	ELK HYPO 2A	7/27/2000	12/11/2003	52										1		
Elk	ELK HYPO 2B	7/27/2000	12/11/2003	47												
Eagle's Nest 2	EN2 EPI 1A	6/8/2000	12/10/2003	68	1		2									
Eagle's Nest 2	EN2 EPI 1B	6/8/2000	12/10/2003	53		3	2									
Eagle's Nest 2	EN2 EPI 2A	6/8/2000	12/10/2003	65	4	2	2									
Eagle's Nest 2	EN2 EPI 2B	6/8/2000	12/10/2003	62	1	2	1									
Eagle's Nest 2	EN2 META 1A	6/8/2000	12/10/2003	56	1	2	1									
Eagle's Nest 2	EN2 META 1B	6/8/2000	12/10/2003	53	3											
Eagle's Nest 2	EN2 META 2A	6/8/2000	12/10/2003	62	2	2										
Eagle's Nest 2	EN2 META 2B	6/8/2000	12/10/2003	60	1		3									
Eagle's Nest 2	EN2 HYPO 1A	6/8/2000	12/10/2003	63	1	1										
Eagle's Nest 2	EN2 HYPO 1B	6/8/2000	12/10/2003	58	1	3										

Table 5 continued.

Lake Name	Lake Identification Code	Sample Date	Count Date	Total Volume	*D. mendotae*			*D. pulicaria*			*D. ambigua*			*D. retrocurva*		
					A	B	C	A	B	C	A	B	C	A	B	C
Farm	FAR EPI 1A	6/7/2000	12/12/2003	65												
Farm	FAR EPI 1B	6/7/2000	12/12/2003	65	2	1										
Farm	FAR EPI 2A	6/7/2000	12/12/2003	67												
Farm	FAR EPI 2B	6/7/2000	12/12/2003	68		1										
Farm	FAR META 1A	6/7/2000	12/12/2003	57						2						
Farm	FAR META 1B	6/7/2000	12/12/2003	68		1	1			1						
Farm	FAR META 2A	6/7/2000	12/12/2003	66			1									
Farm	FAR META 2B	6/7/2000	12/12/2003	50					1							
Farm	FAR HYPO 1A	6/7/2000	12/12/2003	60	1			2								
Farm	FAR HYPO 1B	6/7/2000	12/12/2003	56												
Fenske	FEN EPI 1A	6/9/2000	12/12/2003	49												
Fenske	FEN EPI 1B	6/9/2000	12/12/2003	58			1									
Fenske	FEN EPI 2A	6/9/2000	12/12/2003	53				1		1						
Fenske	FEN EPI 2B	6/9/2000	12/12/2003	58				1		1						
Fenske	FEN META 1A	6/9/2000	12/12/2003	58					1							
Fenske	FEN META 1B	6/9/2000	12/12/2003	52				1								
Fenske	FEN META 2A	6/9/2000	12/12/2003	45		1		1								
Fenske	FEN META 2B	6/9/2000	12/12/2003	70												
Fenske	FEN HYPO 1A	6/9/2000	12/12/2003	53												
Fenske	FEN HYPO 1B	6/9/2000	12/12/2003	63					2	1						
Fenske	FEN HYPO 2A	6/9/2000	12/12/2003	64					1							
Fenske	FEN HYPO 2B	6/9/2000	12/12/2003	50												
Goguac	GOG EPI 1A	6/21/2001	1/5/2004	47												
Goguac	GOG EPI 1B	6/21/2001	1/5/2004	43				2		1				2	1	
Goguac	GOG EPI 2A	6/21/2001	1/5/2004	41				1						3	2	
Goguac	GOG EPI 2B	6/21/2001	1/5/2004	55										2	3	1
Goguac	GOG META 1A	6/21/2001	1/5/2004	48											2	3
Goguac	GOG META 1B	6/21/2001	1/5/2004	47					1	1				1	4	
Goguac	GOG META 2A	6/21/2001	1/5/2004	45											1	1
Goguac	GOG META 2B	6/21/2001	1/5/2004	48						2						1
Goguac	GOG HYPO 1A	6/21/2001	1/5/2004	41										2		
Goguac	GOG HYPO 1B	6/21/2001	1/5/2004	41											1	1
Goguac	GOG HYPO 2A	6/21/2001	1/5/2004	50												
Goguac	GOG HYPO 2B	6/21/2001	1/5/2004	54												

Table 5 continued.

Lake Name	Lake Identification Code	Sample Date	Count Date	Total Volume	*D. mendotae*			*D. pulicaria*			*D. ambigua*			*D. retrocurva*		
					A	B	C	A	B	C	A	B	C	A	B	C
Harriet	HAR EPI 1A	8/14/2000	12/11/2003	53												
Harriet	HAR EPI 1B	8/14/2000	12/11/2003	63												1
Harriet	HAR EPI 2A	8/14/2000	12/11/2003	58		1	1									
Harriet	HAR EPI 2B	8/14/2000	12/11/2003	51												
Harriet	HAR META 1A	8/14/2000	12/11/2003	48												
Harriet	HAR META 1B	8/14/2000	12/11/2003	66	1											
Harriet	HAR META 2A	8/14/2000	12/11/2003	54										1		
Harriet	HAR META 2B	8/14/2000	11/28/2003	57												
Harriet	HAR HYPO 1A	8/14/2000	11/28/2003	59												
Harriet	HAR HYPO 1B	8/14/2000	11/28/2003	52												
Harriet	HAR HYPO 2A	8/14/2000	11/28/2003	54		1										
Harriet	HAR HYPO 2B	8/14/2000	12/11/2003	55												
Hogback	HOG EPI 1A	6/21/2000	11/30/2003	54			1									
Hogback	HOG EPI 1B	6/21/2000	11/30/2003	46			1									
Hogback	HOG EPI 2A	6/21/2000	11/30/2003	45	2											
Hogback	HOG EPI 2B	6/21/2000	11/30/2003	43		2										
Hogback	HOG META 1A	6/21/2000	11/30/2003	43												
Hogback	HOG META 1B	6/21/2000	11/30/2003	47		1										
Hogback	HOG META 2A	6/21/2000	11/30/2003	47												
Hogback	HOG META 2B	6/21/2000	11/30/2003	38												
Hogback	HOG HYPO 1A	6/21/2000	11/30/2003	40												
Hogback	HOG HYPO 1B	6/21/2000	11/30/2003	50												
Hogback	HOG HYPO 2A	6/21/2000	11/30/2003	33	1	1				1						
Hogback	HOG HYPO 2B	6/21/2000	11/30/2003	55		1										

Table 5 continued.

Lake Name	Lake Identification Code	Sample Date	Count Date	Total Volume	*D. mendotae*			*D. pulicaria*			*D. ambigua*			*D. retrocurva*		
					A	B	C	A	B	C	A	B	C	A	B	C
Itasca	ITA EPI 1A	7/19/2000	11/28/2003	40												
Itasca	ITA EPI 1B	7/19/2000	11/28/2003	49												
Itasca	ITA EPI 2A	7/19/2000	11/28/2003	50												
Itasca	ITA EPI 2B	7/19/2000	11/28/2003	40												
Itasca	ITA META 1A	7/19/2000	11/28/2003	48	1		1									
Itasca	ITA META 1B	7/19/2000	11/28/2003	40												
Itasca	ITA META 2A	7/19/2000	11/28/2003	41			1									
Itasca	ITA META 2B	7/19/2000	11/28/2003	50												
Itasca	ITA HYPO 1A	7/19/2000	11/28/2003	43												
Itasca	ITA HYPO 1B	7/19/2000	11/28/2003	45						1						
Itasca	ITA HYPO 2A	7/19/2000	11/28/2003	45												
Itasca	ITA HYPO 2B	7/19/2000	11/28/2003	50												
Josephine	JOS EPI 1A	8/7/2000	12/1/2003	58												1
Josephine	JOS EPI 1B	8/7/2000	12/1/2003	63												
Josephine	JOS EPI 2A	8/7/2000	12/1/2003	56												
Josephine	JOS EPI 2B	8/7/2000	12/1/2003	61												
Josephine	JOS META 1A	8/7/2000	12/1/2003	53												
Josephine	JOS META 1B	8/7/2000	12/1/2003	62												
Josephine	JOS META 2A	8/7/2000	12/1/2003	58												
Josephine	JOS META 2B	8/7/2000	12/1/2003	50										1		
Josephine	JOS HYPO 1A	8/7/2000	12/1/2003	59										1		1
Josephine	JOS HYPO 1B	8/7/2000	12/1/2003	60												
Josephine	JOS HYPO 2A	8/7/2000	12/1/2003	57												
Josephine	JOS HYPO 2B	8/7/2000	12/1/2003	56											1	

Table 5 continued.

Lake Name	Lake Identification Code	Sample Date	Count Date	Total Volume	*D. mendotae*			*D. pulicaria*			*D. ambigua*			*D. retrocurva*		
					A	B	C	A	B	C	A	B	C	A	B	C
Long (LNG)	LNG EPI 1A	6/26/2001	1/6/2004	52												
Long (LNG)	LNG EPI 1B	6/26/2001	1/6/2004	35												
Long (LNG)	LNG EPI 2A	6/26/2001	1/6/2004	44												
Long (LNG)	LNG EPI 2B	6/26/2001	1/6/2004	44												
Long (LNG)	LNG META 1A	6/26/2001	1/6/2004	43												
Long (LNG)	LNG META 1B	6/26/2001	1/6/2004	50												
Long (LNG)	LNG META 2A	6/26/2001	1/6/2004	50												
Long (LNG)	LNG META 2B	6/26/2001	1/6/2004	55												
Long (LNG)	LNG HYPO 1A	6/26/2001	1/6/2004	53				2		1						
Long (LNG)	LNG HYPO 1B	6/26/2001	1/6/2004	60						1						
Long (LNG)	LNG HYPO 2A	6/26/2001	1/6/2004	55						1						
Long (LNG)	LNG HYPO 2B	6/26/2001	1/6/2004	50				1								
Long (LON)	LON EPI 1A	7/12/2000	11/24/2003	57												
Long (LON)	LON EPI 1B	7/12/2000	11/25/2003	58												
Long (LON)	LON EPI 2A	7/12/2000	11/25/2003	44												
Long (LON)	LON EPI 2B	7/12/2000	11/25/2003	42												
Long (LON)	LON META 1A	7/12/2000	11/24/2003	49.5												
Long (LON)	LON META 1B	7/12/2000	11/25/2003	44												
Long (LON)	LON META 2A	7/12/2000	11/25/2003	38												
Long (LON)	LON META 2B	7/12/2000	11/25/2003	42				1								
Long (LON)	LON HYPO 1A	7/12/2000	11/24/2003	48					1							
Long (LON)	LON HYPO 1B	7/12/2000	11/24/2003	49												
Long (LON)	LON HYPO 2A	7/12/2000	11/25/2003	44					1	1						
Long (LON)	LON HYPO 2B	7/12/2000	11/25/2003	42				1								

Table 5 continued.

Lake Name	Lake Identification Code	Sample Date	Count Date	Total Volume	*D. mendotae*			*D. pulicaria*			*D. ambigua*			*D. retrocurva*		
					A	B	C	A	B	C	A	B	C	A	B	C
Many Point	MAN EPI 1A	7/15/2000	12/2/2003	63												
Many Point	MAN EPI 1B	7/15/2000	12/2/2003	58												
Many Point	MAN EPI 2A	7/15/2000	12/2/2003	53												1
Many Point	MAN EPI 2B	7/15/2000	12/2/2003	55										1		1
Many Point	MAN META 1A	7/15/2000	12/2/2003	58											4	1
Many Point	MAN META 1B	7/15/2000	12/2/2003	61										2		3
Many Point	MAN META 2A	7/15/2000	12/2/2003	54										1	1	
Many Point	MAN META 2B	7/15/2000	12/2/2003	51										3		1
Many Point	MAN HYPO 1A	7/15/2000	12/2/2003	50										2		
Many Point	MAN HYPO 1B	7/15/2000	12/2/2003	47										1		
Many Point	MAN HYPO 2A	7/15/2000	12/2/2003	53												
Many Point	MAN HYPO 2B	7/15/2000	12/2/2003	57										1		
Miner	MIN EPI 1A	6/14/2001	1/2/2004	58			1								1	
Miner	MIN EPI 1B	6/14/2001	1/2/2004	57	8	7	5									
Miner	MIN META 1A	6/14/2001	1/2/2004	54	12	2	5			1						
Miner	MIN META 1B	6/14/2001	1/2/2004	57	5	2	1	1								
Miner	MIN META 2A	6/14/2001	1/2/2004	55												
Miner	MIN META 2B	6/14/2001	1/2/2004	50				1								
Miner	MIN HYPO 1A	6/14/2001	1/2/2004	46		1		5	2	3						
Miner	MIN HYPO 1B	6/14/2001	1/2/2004	55		2		1		1						
Miner	MIN HYPO 2A	6/14/2001	1/2/2004	49	1	1			1	2						
Miner	MIN HYPO 2B	6/14/2001	1/2/2004	50			1			2						
Moose	MOS EPI 1A	6/19/2000	12/3/2003	52											1	
Moose	MOS EPI 1B	6/19/2000	12/3/2003	58												
Moose	MOS EPI 2A	6/19/2000	12/3/2003	54												
Moose	MOS EPI 2B	6/19/2000	12/3/2003	58											1	
Moose	MOS META 1A	6/19/2000	12/3/2003	48												1
Moose	MOS META 1B	6/19/2000	12/3/2003	59											1	
Moose	MOS META 2A	6/19/2000	12/3/2003	44												
Moose	MOS META 2B	6/19/2000	12/16/2003	64			1									
Moose	MOS HYPO 1A	6/19/2000	12/16/2003	54		1										
Moose	MOS HYPO 1B	6/19/2000	12/16/2003	48												
Moose	MOS HYPO 2A	6/19/2000	12/16/2003	48		2										
Moose	MOS HYPO 2B	6/19/2000	12/16/2003	47	2											

Table 5 continued.

Lake Name	Lake Identification Code	Sample Date	Count Date	Total Volume	*D. mendotae*			*D. pulicaria*			*D. ambigua*			*D. retrocurva*		
					A	B	C	A	B	C	A	B	C	A	B	C
Minnetonka	MTK EPI 1A	8/15/2000	12/2/2003	45										3		7
Minnetonka	MTK EPI 1B	8/15/2000	12/2/2003	67										2	3	3
Minnetonka	MTK EPI 2A	8/15/2000	12/2/2003	56											1	1
Minnetonka	MTK EPI 2B	8/15/2000	12/2/2003	50											1	1
Minnetonka	MTK META 1A	8/15/2000	12/2/2003	51	1				1	2						1
Minnetonka	MTK META 1B	8/15/2000	12/2/2003	52			1	1								
Minnetonka	MTK META 2A	8/15/2000	12/2/2003	51										2		
Minnetonka	MTK META 2B	8/15/2000	12/2/2003	58										1		
Minnetonka	MTK HYPO 1A	8/15/2000	12/2/2003	48										1		1
Minnetonka	MTK HYPO 1B	8/15/2000	12/2/2003	49										1		3
Newman	NEW EPI 1A	7/23/2000	12/16/2003	44												
Newman	NEW EPI 1B	7/23/2000	12/16/2003	47												
Newman	NEW EPI 2A	7/23/2000	12/16/2003	57		3	1									
Newman	NEW EPI 2B	7/23/2000	12/16/2003	48		1										
Newman	NEW META 1A	7/23/2000	12/16/2003	48		2	1									
Newman	NEW META 1B	7/23/2000	12/16/2003	48		1										
Newman	NEW META 2A	7/23/2000	12/16/2003	65	1	1	1									
Newman	NEW META 2B	7/23/2000	12/16/2003	49	1		1									
Newman	NEW HYPO 1A	7/23/2000	12/16/2003	51	2		1									
Newman	NEW HYPO 1B	7/23/2000	12/16/2003	53												
Newman	NEW HYPO 2A	7/23/2000	12/16/2003	56												
Newman	NEW HYPO 2B	7/23/2000	12/16/2003	52		1										
Okoboji	OKO EPI 1A	5/25/2000	12/24/2003	53				3	1	4						
Okoboji	OKO EPI 1B	5/25/2000	12/24/2003	56				1	2	2						
Okoboji	OKO EPI 2A	5/25/2000	12/24/2003	62						1						
Okoboji	OKO EPI 2B	5/25/2000	12/24/2003	52												
Okoboji	OKO META 1A	5/25/2000	12/24/2003	45				3								
Okoboji	OKO META 1B	5/25/2000	12/24/2003	53				1								
Okoboji	OKO META 2A	5/25/2000	12/24/2003	48						1						
Okoboji	OKO META 2B	5/25/2000	12/24/2003	56				2	2	1						
Okoboji	OKO HYPO 1A	5/25/2000	12/24/2003	48				2	1							
Okoboji	OKO HYPO 1B	5/25/2000	12/24/2003	56				1	2	1						
Okoboji	OKO HYPO 2A	5/25/2000	12/24/2003	39												
Okoboji	OKO HYPO 2B	5/25/2000	12/24/2003	59				1								

Table 5 continued.

Lake Name	Lake Identification Code	Sample Date	Count Date	Total Volume	*D. mendotae*			*D. pulicaria*			*D. ambigua*			*D. retrocurva*		
					A	B	C	A	B	C	A	B	C	A	B	C
Plantagenet	PLA EPI 1A	7/24/2000	12/19/2003	55	1				2							
Plantagenet	PLA EPI 1B	7/24/2000	12/19/2003	58												
Plantagenet	PLA EPI 2A	7/24/2000	12/19/2003	66												
Plantagenet	PLA EPI 2B	7/24/2000	12/19/2003	62				1								
Plantagenet	PLA META 1A	7/24/2000	12/19/2003	46		1										
Plantagenet	PLA META 1B	7/24/2000	12/19/2003	46												
Plantagenet	PLA META 2A	7/24/2000	12/19/2003	48												
Plantagenet	PLA META 2B	7/24/2000	12/19/2003	43	1											
Plantagenet	PLA HYPO 1A	7/24/2000	12/19/2003	46			1									
Plantagenet	PLA HYPO 1B	7/24/2000	12/19/2003	48												
Plantagenet	PLA HYPO 2A	7/24/2000	12/19/2003	42												
Plantagenet	PLA HYPO 2B	7/24/2000	12/19/2003	39												
Little Sand	SAN EPI 1A	7/20/2000	12/1/2003	51												
Little Sand	SAN EPI 1B	7/20/2000	12/1/2003	50												
Little Sand	SAN EPI 2A	7/20/2000	12/1/2003	49												
Little Sand	SAN EPI 2B	7/20/2000	12/1/2003	36							1					
Little Sand	SAN META 1A	7/20/2000	12/1/2003	48								2	1			
Little Sand	SAN META 1B	7/20/2000	12/1/2003	50							2		1			
Little Sand	SAN META 2A	7/20/2000	12/1/2003	58								1	1			
Little Sand	SAN META 2B	7/20/2000	12/1/2003	56												
Little Sand	SAN HYPO 1A	7/20/2000	12/1/2003	58							1	2	1			
Little Sand	SAN HYPO 1B	7/20/2000	12/1/2003	60								1				
Little Sand	SAN HYPO 2A	7/20/2000	12/1/2003	65												
Little Sand	SAN HYPO 2B	7/20/2000	12/1/2003	47												

Table 5 continued.

Lake Name	Lake Identification Code	Sample Date	Count Date	Total Volume	*D. mendotae*			*D. pulicaria*			*D. ambigua*			*D. retrocurva*		
					A	B	C	A	B	C	A	B	C	A	B	C
Snowbank	SNO EPI 1A	6/15/2000	12/22/2003	55	1		1									
Snowbank	SNO EPI 1B	6/15/2000	12/22/2003	53												
Snowbank	SNO EPI 2A	6/15/2000	12/22/2003	53			1									
Snowbank	SNO EPI 2B	6/15/2000	12/22/2003	54												
Snowbank	SNO META 1A	6/15/2000	12/22/2003	66	1	1										
Snowbank	SNO META 1B	6/15/2000	12/22/2003	52		1		1								
Snowbank	SNO META 2A	6/15/2000	12/22/2003	45												
Snowbank	SNO META 2B	6/15/2000	12/22/2003	46	1	1										
Snowbank	SNO HYPO 1A	6/15/2000	12/22/2003	54				1								
Snowbank	SNO HYPO 1B	6/15/2000	12/22/2003	61												
Snowbank	SNO HYPO 2A	6/15/2000	12/22/2003	56												
Snowbank	SNO HYPO 2B	6/15/2000	12/22/2003	45												
Upper Bottle	UPB EPI 1A	7/14/2000	12/24/2003	50												
Upper Bottle	UPB EPI 1B	7/14/2000	12/24/2003	51												
Upper Bottle	UPB EPI 2A	7/14/2000	12/24/2003	51												
Upper Bottle	UPB EPI 2B	7/14/2000	12/24/2003	56												
Upper Bottle	UPB META 1A	7/14/2000	12/24/2003	40												
Upper Bottle	UPB META 1B	7/14/2000	12/24/2003	50												
Upper Bottle	UPB META 2A	7/14/2000	12/24/2003	41												
Upper Bottle	UPB META 2B	7/14/2000	12/24/2003	55												
Upper Bottle	UPB HYPO 1A	7/14/2000	12/24/2003	52			3									
Upper Bottle	UPB HYPO 1B	7/14/2000	12/24/2003	50												
White Bear	WBL EPI 1A	8/9/2000	12/23/2003	44										3	2	1
White Bear	WBL EPI 1B	8/9/2000	12/23/2003	50						1				7	1	2
White Bear	WBL EPI 2A	8/9/2000	12/23/2003	53										2	4	
White Bear	WBL EPI 2B	8/9/2000	12/23/2003	55										1	1	2
White Bear	WBL META 1A	8/9/2000	12/23/2003	52										1	1	1
White Bear	WBL META 1B	8/9/2000	12/23/2003	49											2	
White Bear	WBL META 2A	8/9/2000	12/23/2003	54												
White Bear	WBL META 2B	8/9/2000	12/23/2003	52										1	2	
White Bear	WBL HYPO 1A	8/9/2000	12/23/2003	56												1
White Bear	WBL HYPO 1B	8/9/2000	12/23/2003	50										2		1
White Bear	WBL HYPO 2A	8/9/2000	12/23/2003	50												
White Bear	WBL HYPO 2B	8/9/2000	12/23/2003	46												1

Table 5 continued.

Lake Name	Lake Identification Code	Sample Date	Count Date	Total Volume	*D. mendotae*			*D. pulicaria*			*D. ambigua*			*D. retrocurva*		
					A	B	C	A	B	C	A	B	C	A	B	C
Christmas	XMS EPI 1A	8/12/2000	12/10/2003	46					1					1	2	1
Christmas	XMS EPI 1B	8/12/2000	12/10/2003	53			1									2
Christmas	XMS EPI 2A	8/12/2000	12/10/2003	49			1									
Christmas	XMS EPI 2B	8/12/2000	12/10/2003	51										2		1
Christmas	XMS META 1A	8/12/2000	12/10/2003	49			1									
Christmas	XMS META 1B	8/12/2000	12/10/2003	43				2						1		
Christmas	XMS META 2A	8/12/2000	12/10/2003	60										1		
Christmas	XMS META 2B	8/12/2000	12/10/2003	52	1					1				1		
Christmas	XMS HYPO 1A	8/12/2000	12/10/2003	50			1		1							
Christmas	XMS HYPO 1B	8/12/2000	12/10/2003	53					1	1						
Christmas	XMS HYPO 2A	8/12/2000	12/10/2003	55											1	
Christmas	XMS HYPO 2B	8/12/2000	12/10/2003	45												