COMMUNITY STRUCTURE AND AVAILABILITY OF EDIBLE PRIMARY PRODUCERS TO HERBIVORES ALONG A TEMPERATURE GRADIENT

by

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Abstract

Warming can influence the rate of plant-herbivore interactions through direct effects on individual metabolism, resource use, and growth rates, and via indirect effects on the properties of plant resources and behavior of consumers. Through these processes, temperature can affect the structure and function of food webs, though whether these overall responses reflect primarily direct or indirect effects of temperature is unclear. To begin to address this problem, I quantified the effects of temperature and grazing on primary producer traits and relative abundance to understand how temperature directly and indirectly affects an important aspect of food webs: resource availability to herbivores. I hypothesized that warming would decrease the availability of edible resources to consumers through decreased abundance, body size and shifts among dominant functional groups, and that these effects would be strengthened in the presence of consumers. I tested this hypothesis in freshwater algal-grazer communities maintained across an 11°C temperature gradient over 11 weeks. I observed direct, positive effects of temperature on whole-system oxygen fluxes (i.e. through net primary productivity and ecosystem respiration), and direct negative effects on phytoplankton abundance and body size, with higher relative abundance of small phytoplankton. Herbivores drove shifts in phytoplankton size distributions across the temperature gradient through size-selective consumption of large phytoplankton. Warming shifted species composition among algae from plankton-dominated to periphyton-dominated assemblages, consistent with indirect effects of warming on competitive interactions. Taken together, shifts in abundance, body size and functional group dominance over the temperature gradient decreased the availability of preferred plant resources to filter-feeding zooplankton at warmer temperatures, which may alter food web structure and function, especially under increased grazing pressure. I conclude that resource-availability shifts are predictable with warming, and that temperature-dependent community theory can be expanded to include these indirect effects of temperature on species interactions.
Preface

This thesis is original, unpublished work by the author, N. C. Caulk.
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Dedication

For my family: Katie, Bob, Bruno, and Jenny.
Chapter 1

Introduction

Individual variation in consumption, growth, and reproduction are partially explained by the effects of temperature on organismal physiology. On the organismal level, metabolic rate scales directly with temperature and body size, an observation which forms the basis for the metabolic theory of ecology (MTE) (Gillooly et al. 2001; Brown et al. 2004). The metabolic theory of ecology provides a framework for understanding how temperature-dependent physiological responses of organisms affect broad ecological patterns like carbon and nutrient cycling, and species diversity (Algar et al. 2007; Allen and Gillooly 2009; Schramski et al. 2015). Despite this theoretical framework, empirical tests of MTE at the community-level remain extremely challenging for ecologists.

Scaling-up organismal-level temperature responses to multi-species interactions is complicated by asymmetrical thermal sensitivities of interacting organisms. Such asymmetries can strengthen or weaken trophic interactions, depending on the direction of the temperature shift and the foraging strategy of the consumer (Sanford 1999; Morelissen and Harley 2007; O’Connor et al. 2009; Dell et al. 2013). Understanding temperature-dependencies of consumer-resource interactions is extremely important, since these interactions determine the flux of nutrients and materials within and among ecosystems.

Plant-herbivore interactions regulate the amount of primary productivity within a system and are present in most food webs. Previous research has shown a general strengthening of plant-herbivore interactions with increasing temperature, due to higher temperature-sensitivities of heterotrophic metabolism than autotrophic metabolism (Allen et al. 2005; López-Urrutia et al. 2006; O’Connor 2009). However, metabolic scaling does not account for indirect effects of temperature on properties of primary producers or consumer behavior, which may change the predicted effects of warming on plant-herbivore interaction strength.

Temperature can alter the traits of primary producers via changes in chemical defenses (Sudatti et al. 2011; Weinberger et al. 2011) and nutritional quality (Staehr and Wernberg
Such changes impact the palatability of resources, and subsequently alter plant-herbivore interactions. Further, feeding preferences of herbivores can vary with temperature, even when plant resources remain unchanged (Sotka and Giddens 2009). Warming-induced shifts in body size of either plants or herbivores have important implications for interactions between size-selective consumers and their prey. Finally, temperature may increase or decrease the abundance of preferred resource types. Identifying and understanding potential indirect effects of temperature on consumer-resource interactions is important to make accurate predictions about the long-term impact of temperature on food web dynamics.

1.1 Study system

Phytoplankton form the base of aquatic food webs and play a large role in global carbon flux, contributing to approximately half of primary production on Earth (Field et al. 1998). Phytoplankton production is dependent upon light, nutrients, and temperature, but top-down forces also strongly limit phytoplankton abundance (Field et al. 1998; Lewandowska et al. 2012; Shurin et al. 2012). Grazing by zooplankton alters phytoplankton biomass, body size, and species diversity (Brooks and Dodson 1965; McCauley and Briand 1979; Sommer and Lewandowska 2011).

Trophic dynamics in aquatic systems are highly size-structured. Herbivorous filter-feeding zooplankton are primarily generalists and limited to the consumption of particles that do not exceed their mouth gape. Of particular importance within freshwater systems are *Daphnia*, large cladoceran zooplankton, which feed efficiently on both large and small phytoplankton (Peterson et al. 1978; DeMott 1982). In the absence of predation by fish, *Daphnia* are able to diminish phytoplankton populations (Brooks and Dodson 1965; Kratina et al. 2012). High fish predation on *Daphnia* generates a trophic cascade, whereby phytoplankton standing stocks increase due to decreased grazing pressure by *Daphnia*, which are replaced by smaller, less-efficient filter-feeders (Brooks and Dodson 1965).

*Daphnia* act as a keystone species, maintaining phytoplankton diversity by grazing preferentially upon fast-growing phytoplankton species that would otherwise be superior competitors for light and nutrients (McCauley and Briand 1979; Sarnelle 2005). However,
bottom-up forces driving phytoplankton assemblages can subsequently affect *Daphnia* populations. Zooplankton population growth is dependent upon the timing, magnitude, and composition of the spring phytoplankton bloom, which are determined both by abiotic growth conditions and densities of overwintering zooplankton (Sommer and Lengfellner 2008; Sommer and Lewandowska 2011). Resulting post-bloom phytoplankton assemblages are differentially edible to zooplankton like *Daphnia*, shifting zooplankton herbivore assemblages towards those which can feed most efficiently on the abundant phytoplankton species (Dupuis and Hann 2009).

Thus, both bottom-up and top-down forces on phytoplankton interact to generate feedbacks on zooplankton populations, and these interactions are likely to change with varying abiotic pressure on ecosystems. Warming has been linked to global phytoplankton declines (Boyce et al. 2010; Lewandowska et al. 2014), and also directly affects the diversity and feeding rates of zooplankton (Burns 1969; Steiner 2004; Dupuis and Hann 2009). The resulting changes in trophic interactions between phytoplankton and zooplankton with warming will have important consequences for the structure and function of aquatic food webs.

### 1.2 Structure & objectives

The goal of this thesis is to quantify the effects of temperature and consumers on modifying the abundance and traits of primary producer assemblages, with implications for the availability of plant resources in freshwater systems. This thesis consists of three chapters: an introduction, a data chapter, and a concluding chapter. In chapter two, I present the results of a warming experiment, in which I manipulated temperature and trophic structure within freshwater mesocosms to answer the following questions: 1) How do primary producer community responses (abundance, body size, oxygen fluxes) vary with temperature and the presence or absence of consumers? 2) Does warming act to decrease the availability of preferred resources to consumers? Chapter 3 concludes this thesis by exploring the implications of my findings for food web structure in aquatic systems and proposing directions for future research in this field.
Chapter 2

Estimating the Availability of Edible Plant Resources across a Temperature Gradient

2.1 Introduction

Temperature determines the rate of consumer-resource interactions, with profound implications for trophic dynamics and food web structure (Sanford 1999; O’Connor et al. 2009; Dell et al. 2013). Plant-herbivore interactions form a basic module of most food webs, and provide a foundation for understanding how differential metabolic scaling of organisms impacts consumer-resource dynamics. Heterotrophic (respiration-limited) metabolism is more sensitive to temperature than autotrophic (photosynthesis-limited) metabolism, and this asymmetry in temperature dependence can shift plant-herbivore dynamics toward a strengthening of consumer control over plants at increased temperatures (Allen et al. 2005; López-Urrutia et al. 2006; O’Connor 2009; O’Connor et al. 2011; Gilbert et al. 2014). However, these direct effects of temperature on herbivore-plant interactions could be complicated by indirect effects of temperature. Indirect effects of temperature, from the perspective of a focus on the herbivore-plant interaction, include changes in the availability of resources, changes in consumer feeding preferences, or changes in resource quality, in turn altering the outcomes of herbivore-plant interactions in ways that amplify or obscure the metabolic effects of temperature. Such feedbacks are unaccounted for in the current framework of temperature-dependent consumer-resource dynamics, which relies primarily on metabolic scaling predictions to understand the effects of warming on consumer-resource interactions.

The metabolic theory of ecology (MTE) states that rates of photosynthesis increase predictably with temperature (Gillooly et al. 2001; Brown et al. 2004), and consequently, when resources are available, so does plant and algal population growth, carbon fixation and primary productivity (Eppley 1972; Allen et al. 2005; Falkowski and Raven 2007; Bissinger et al. 2008). However, effects of warming-induced increases in photosynthesis are not always apparent at the population or assemblage-level, instead primary productivity is lower or
constant with warming (Behrenfeld et al. 2006; Enquist et al. 2007; Lewandowska et al. 2012). Temperature effects on phytoplankton primary productivity are mediated by light, nutrients and lethal temperature effects on phytoplankton survival, resulting in changes in phytoplankton assemblages which may indirectly affect plant-herbivore interactions with warming. In pelagic food webs, decreases in phytoplankton abundance can drive declines in community biomass at warmer temperatures, despite greater primary productivity (O’Connor et al. 2009; Yvon-Durocher et al. 2011). Warming promotes phytoplankton losses via increased sinking and respiration (Falkowski and Raven 2007; Finkel et al. 2009), and biomass declines may be intensified by warming-induced decreases in phytoplankton body size (Atkinson et al. 2003; Yvon-Durocher et al. 2011). Accelerated metabolic rates with warming enhance nutrient uptake, effectively heightening resource limitation at higher temperatures (Gillooly et al. 2001; Allen and Gillooly 2009) and potentially limiting biomass accumulation. Indeed, global declines in oceanic phytoplankton are linked to both temperature and nutrient limitation (Doney 2006; Boyce et al. 2010).

Warming-induced shifts in body size may contribute to temperature-driven changes in primary producer abundance and biomass, further obscuring the link between direct effects of temperature on photosynthesis and resulting community structure. Generally, the relative abundance of small organisms increases with warming, with a decrease in large organisms across community types (Yvon-Durocher et al. 2011; Dossena et al. 2012). Yvon-Durocher et al. (2011) concluded that shifts in phytoplankton body size with warming were primarily due to increased relative abundance of small phytoplankton species, and not warming-induced declines in body size as predicted by the temperature size rule (Atkinson 1994; Atkinson and Sibly 1997). Shifts in the body size and community structure of phytoplankton are important for determining the strength of trophic interactions with size-selective consumers.

The effect of temperature on primary producers depends on photosynthetic rates, the phenotype (i.e. thermal niche, body size) of the particular organisms involved, rates and outcomes of species interactions (i.e. grazing and competition), and nutrient availability. Thus, the community-level effects of temperature on primary producer abundance often diverge from direct extensions of the effect of temperature on photosynthesis and instead reflect the balance of these factors. For example, in one study, despite strong evidence for declining
productivity and producer biomass in pelagic systems with warming, warming did not significantly reduce community biomass, on average, in benthic aquatic communities (Dossena et al. 2012). Rather, standing biomass of attached algae (periphyton) is expected to increase with warmer temperatures in the absence of consumers (Hillebrand 2009; Shurin et al. 2012; Cao et al. 2014; O’Regan et al. 2014). The ability of attached algae to increase in abundance with warming is likely driven in part by superior competitive ability for nutrient uptake over phytoplankton (Fong et al. 1993; Blumenshine et al. 1997). Thus, the effects of warming on aquatic food webs may not be fully captured when investigating pelagic or benthic communities alone. From the perspective of herbivores, differential shifts in the availability of these two plant resource types have important consequences for individual and population growth. Few studies have investigated the effects of warming on pelagic and benthic primary producers together (Shurin et al. 2012). Further, the majority of experimental warming studies lack consumer-free treatments and are therefore unable to interpret the effects of warming on primary producers in the absence of grazing.

Herbivory may strengthen or reverse temperature-induced changes in traits, density, and productivity of primary producer assemblages. Size-selective consumption by zooplankton alters the size distribution of phytoplankton communities (Katechakis et al. 2002), and warming-induced strengthening of this consumer-pressure can amplify declines in abundance and phytoplankton body size (O’Connor et al. 2009; Sommer and Lewandowska 2011). In benthic communities, the positive effect of warming on attached algal biomass may be absent or reversed with consumer presence (Hillebrand 2009; Shurin et al. 2012; Cao et al. 2014; O’Regan et al. 2014). Thus, indirect effects of consumers can change the outcome of the direct temperature-effects which structure primary producer assemblages.

Not only can changes in the body size and abundance of primary producers reflect herbivore or resource responses to temperature, they can also change the availability of primary producers to herbivores. Phenotypic or density responses to temperature could therefore alter food web dynamics and community structure over a temperature gradient by altering herbivory from the bottom-up. To capture these effects, here we use the term ‘availability’ of plants to herbivores to describe whether or not consumable plant resources are present to a
consumer. Specifically, consumable plant resources are those which are accessible, palatable, and edible to consumers.

Here, I test whether direct and indirect effects of warming can decrease preferred resource availability to consumers. I used a freshwater zooplankton and algal species assemblage to quantify the responses of primary producer abundance, traits, and ecosystem fluxes over a temperature gradient (approximately 20-30°C). Specifically, I tested the following hypotheses: 1) Phytoplankton abundance and body size decline with increasing temperature despite increasing ecosystem primary productivity, 2) the strength of this biomass decline increases in the presence of consumers, and 3) relative abundance of available algal functional groups decreases with warming. These questions will contribute to resolving the potential feedbacks between temperature and herbivores on the availability of plant resources, and the implication of these feedbacks for altering plant-herbivore dynamics.

2.2 Methods

2.2.1 Study system

Phytoplankton form the base of most simple freshwater food webs, acting as a primary food source for herbivorous filter-feeding zooplankton. Phytoplankton communities are structured by bottom-up (temperature, light, nutrients) and top-down (grazing) forces. Large-bodied zooplankton like the freshwater cladoceran *Daphnia* spp. can dramatically shift the structure of phytoplankton assemblages through grazing, often acting as keystone consumers on phytoplankton within freshwater systems (McCauley and Briand 1979; Sarnelle 2005). *Daphnia* are generalists, and, due to their body size, can feed both on very large phytoplankton particles and also on very small bacteria with mean volumes as low as 0.05 μm³ (Peterson et al. 1978; DeMott 1982). Due to their filter-feeding mechanism, *Daphnia* consume primarily suspended particles in the water column, although certain species have been shown to feed upon periphytic algae under very low phytoplankton concentrations (Siehoff et al. 2009). *Daphnia* are known to increase consumption rates with temperature, with the potential to decrease phytoplankton biomass at higher rates with warming (Burns 1969). *Daphnia* may experience a daily temperature range of 10-20°C due to vertical
migration, and a seasonal range of 4-25°C in the surface waters of temperate lakes (Wetzel 2001).

Freshwater plankton systems have a seasonal spring bloom driven by seasonal shifts in light, temperature and nutrient supply. Following a bloom of primary productivity, grazers increase in abundance, and then systems stabilize due to nutrient limitation. The magnitude, timing and composition of phytoplankton spring blooms are known to be altered by climate warming (Sommer and Lengfellner 2008). Here, I am interested in the post-bloom state of this food web to gain insight to the effects of temperature and trophic structure on longer-term, stabilized communities, while minimizing effects of transient population and community dynamics.

2.2.2 Mesocosm experimental design

I experimentally manipulated temperature to test the response of consumers and plant resources in 30 opaque 370-L mesocosms over a gradient of 11°C. All mesocosms were inoculated with a phytoplankton assemblage from Trout Lake, Vancouver, B.C. Lake water was filtered through a 64 μm mesh sieve to include algae smaller than 64 μm and exclude zooplankton and their larvae. One liter of sieved lake water was added to each mesocosm on May 2, 2014, along with a one-time nutrient addition of 100mg NaNO₃ and 6.25mg KH₂PO₄ to stimulate primary production. Half of the mesocosms also received the herbivorous zooplankton *Daphnia pulex* from the UBC experimental pond facility (Figure 2.1). Thirty adult *Daphnia* were added to consumer-treatment mesocosms on May 13, 2014.

Mesocosms were arranged on a gravel pad at the University of British Columbia, Vancouver, Canada (Figure 2.1). Mesocosms were filled with Vancouver municipal water and allowed to sit for one week prior to the addition of organisms, to allow chlorine to evaporate (Kratina et al. 2012). A circular window-screen covered each mesocosm for the duration of the experiment to minimize colonization by insect larvae. Submersible aquarium heaters (50, 100, 150, 200, 250, 300, 350, 400W) were placed in the bottom of each mesocosm to maintain a constant temperature increase above ambient daily temperature. For un-warmed mesocosms, a non-functioning heater was placed on the bottom of the tank to control for any possible effects of heater presence. Water levels were maintained throughout the experiment through natural
precipitation events and additions of dechlorinated city water, as necessary. Temperature was monitored at 30-minute intervals using Thermochron iButtons (Dallas Instruments) submerged to a mid-point in the mesocosms. Treatments were arranged randomly in space.

Figure 2.1. (Left) Experiment was performed in outdoor freshwater mesocosms at the University of British Columbia, Vancouver, Canada. (Right) *Daphnia pulex* were used as herbivores in consumer-treatment mesocosms.

During the 11-week experimental period, I estimated key biological, physical and chemical variables within each mesocosm. I sampled weekly for water column chlorophyll-a, phytoplankton, zooplankton, and oxygen flux (net primary productivity and ecosystem respiration). To compare short-term (immediately post bloom) and long-term states of the communities, I used chlorophyll-a and zooplankton abundance data to identify an early and later time period for intensive community-level sample analysis to test my hypotheses.

### 2.2.3 Estimating fluxes

I measured dissolved oxygen (DO) using a YSI Pro2030 oxygen sensor (Yellow Springs Instruments, Yellow Springs, Ohio, USA). The change in DO over 24 hours was used to estimate whole system net primary productivity (NPP) and ecosystem respiration (ER) (Marzolf et al. 1994). Each week, dissolved oxygen concentration (mg/L) was measured at dawn, dusk, and dawn on the following day. Dawn represents the photosynthetic oxygen minimum, and therefore lowest O₂ levels, with all subsequent DO measurements being higher.
Dusk represents the photosynthetic oxygen maximum, and therefore highest O\textsubscript{2} levels, with all subsequent measurements being lower (Yvon-Durocher et al. 2010; Kratina et al. 2012).

\[
\text{NPP (mgO}_2\text{ L}^{-1}\text{ hr}^{-1}) = (\text{DO}_\text{dusk} - \text{DO}_\text{dawn1}) / \text{hours of measurement duration}
\]

\[
\text{ER (mgO}_2\text{ L}^{-1}\text{ hr}^{-1}) = (\text{DO}_\text{dawn2} - \text{DO}_\text{dusk}) / \text{hours of measurement duration}
\]

Mass-specific net primary productivity was estimated by dividing NPP by phytoplankton biomass, as approximated by chlorophyll-a. This estimate is hereafter referred to as chlorophyll-a-specific NPP (mgO\textsubscript{2} L\textsuperscript{-1} hr\textsuperscript{-1} Chl-a\textsuperscript{-1}). In week 11 only, after considerable algal growth, mass-specific NPP was calculated by dividing NPP by total algal biomass (phytoplankton and attached algae), hereafter referred to as total biomass-specific NPP (mgO\textsubscript{2} L\textsuperscript{-1} hr\textsuperscript{-1} g C\textsuperscript{-1}).

### 2.2.4 Estimating abundance and biomass

Two metrics, water column chlorophyll-a concentration and estimated cell biovolume, are standard proxies for phytoplankton abundance in aquatic research. Often an assumption is made that chlorophyll-a concentration and cell biovolume are directly related. However, chlorophyll-a content per unit biovolume fluctuates with season, light, water depth, nutrients, species composition, cell physiological conditions, and temperature (Riemann et al. 1989; Felip and Catalan 2000; Chen et al. 2011). Thus, I considered both water-column chlorophyll-a concentration and total biovolume. Chlorophyll-a estimates photosynthetically active biomass, while biovolume estimates the total of cell sizes.

Each week, chlorophyll-a concentration was assessed from a 100ml water sample taken approximately 40cm below the surface in each mesocosm. I vacuum-filtered water samples using Whatman GF/C filters and placed each filter into 20ml of 90% acetone for 24 hours of cold-extraction (EPA 2004). I analyzed chlorophyll-a concentration using the non-acidified chlorophyll-a module on a Trilogy Fluorometer (Turner Designs, Sunnyvale, CA). Carbon content of phytoplankton was estimated using the conversion factor of 55 mg C per mg chlorophyll-a (Gasol et al. 1997).

I estimated biovolume (um\textsuperscript{3}) of phytoplankton using a Flow Cytometer and Microscope (FlowCAM). Preserved samples were analyzed in autoimage mode at x100 magnification.
(x10 objective with 100 μm flow cell for 1ml of sample). All particles greater than or equal to 1μm in area based diameter (ABD) were imaged. Images of algal cells were visually separated from detritus, and further divided into broad taxonomic categories. Biovolume was calculated using the appropriate geometric shape of the organism (Hillebrand et al. 1999) with dimensions determined by the FlowCAM. Cell volume was corrected for shrinkage due to Lugol’s preservation (Montagnes et al. 1994).

Biomass of benthic algae and the periphyton growing on mesocosm sides was assessed in the final week of the experiment. Ceramic tiles were placed at the bottom of mesocosms early in the experiment (June 5, 2014). On the final experimental day (August 1, 2014), benthic tiles were removed from the mesocosms and algal biomass was scraped and dried at 60°C for several days to assess dry mass. Periphyton was scraped from the mesocosm sides using plastic blades, filtered onto Whatman GF/C filters and dried at 60°C for several days to assess dry mass. Carbon content of benthic and periphytic algae was estimated assuming that carbon accounted for approximately 25% of biomass, although only 8.4% of periphytic carbon may actually be attributed to algal cells, on average (Frost et al. 2005).

Zooplankton densities were sampled weekly using an 8 inch diameter 63um vertical plankton tow in each tank. Zooplankton were preserved in Lugol’s iodine solution for quantification on a Leica stereoscope.

### 2.2.5 Quantifying algal community structure

Phytoplankton cell sizes were calculated by converting individual biovolume estimates into carbon assuming a conversion factor of 0.109 (Montagnes et al. 1994). I described the size distributions of phytoplankton communities with size spectra constructed by dividing the range of \( \log_{10} \) body size into 6 bins of equal width. Bin width was chosen to maximize linearity and minimize the number of empty bins. The \( \log_{10} \) total abundance of phytoplankton with \( \log_{10} \) body size in each bin was regressed against the center of each bin (White et al. 2008). The slope of the size spectrum describes the decline in abundance with increasing body size.

I used ratios of carbon biomass among algal functional groups to estimate differences in primary producer community structure. Phytoplankton represents the available portion of the
algal community to filter-feeding zooplankton, and as such I used the proportion of total algal biomass as phytoplankton to estimate the availability of algae to zooplankton within the system.

### 2.2.6 Statistical analyses

To characterize bloom dynamics and test predictions about temperature on primary productivity, I used mixed effects models to determine the effects of temperature and time on chlorophyll-a concentrations and oxygen flux (total NPP and ecosystem respiration) across all experimental weeks. Models were compared using Akaike Information Criterion (AIC) and likelihood ratio tests.

I used regression analysis (ANOVA and ANCOVA) to test for effects of temperature and grazers on the following plankton responses: chlorophyll-a concentration; total phytoplankton biovolume; phytoplankton body size; phytoplankton size spectrum slopes and intercepts; and total and mass-specific net primary productivity. I analyzed effects of treatments at two points in time capturing post-bloom and longer-term states. In the final week, I also analyzed effects on periphyton and total algal biomass. For each timepoint, the temperature used in analyses was the average temperature for a particular mesocosm over the previous 7 days, as measured at 30 min intervals by iButton dataloggers. Phytoplankton biovolume estimates were log$_{10}$-transformed to meet the assumptions (normality) of linear regression analysis. Proportion of phytoplankton biomass to total algal biomass was analyzed using linear models with arcsine transformation. All analyses were conducted using R statistical software.

### 2.3 Results

#### 2.3.1 Effects of warming on community responses through time

A bloom characteristic of early transient dynamics of planktonic systems was apparent in all mesocosms in the first week. We identified week 4 as suitable for testing our hypotheses because by this time the bloom had subsided and consumers were persisting in most mesocosms (Figure 2.2a-b). Although total *Daphnia* population abundance peaked in week 5, this was spread among fewer mesocosms (Figure 2.2b). Thus, week 4 maximized total
Daphnia population size and number of mesocosms (n=10) with Daphnia present. We tested for the possibility of an interaction between temperature and the presence or absence of Daphnia (n=10 mesocosms with Daphnia, n=15 without Daphnia) using ANCOVA with type I sums of squares, and if there was no interaction we proceeded with ANCOVA with type II sums of squares, to accommodate the difference in sample size. We analyzed long-term responses in week 11. Because few mesocosms had surviving Daphnia populations in week 11, we analyzed algal assemblages only in mesocosms with no prior Daphnia colonization (n=15).

Average experimental temperature over the 11 week experiment ranged from 19.3°C in the coolest mesocosm to 30.7°C in the warmest mesocosm. Differences among temperatures were maintained while mesocosms tracked daily and weekly variation in temperature (Figure 2.2c). Ambient mesocosms (i.e. mesocosms with no functional heater) consistently differed from high temperature mesocosms by approximately 11°C on average throughout the duration of the experiment (Figure 2.2c). Several mesocosms exhibited anomalous temperature trajectories due to heater failure. Outcome of analyses performed with and without these mesocosms exhibited no meaningful differences, and thus they were included in all analyses. In week 4, average temperature was approximately 2.3°C lower than the average temperature in week 11.
Figure 2.2. (a) Chlorophyll-a peaked after the first experimental week and subsequently declined, on average, across all experimental weeks. Bars represent standard error. (b) Average *Daphnia* abundance per liter (filled circles) and total number of mesocosms with surviving *Daphnia* (open triangles) between initial *Daphnia* addition (week 0) and week 11. Bars represent standard error. *Daphnia* abundance was lowest in high temperature mesocosms, and extinctions continued steadily within mesocosms across all weeks. (c) Average temperature within each mesocosm across all weeks as measured by iButton dataloggers.
Warming increased rates of community NPP and respiration throughout the experiment (Figure 2.3a-b, Table 2.1). NPP increased with increasing temperature, but total NPP and the rate of the increase with temperature varied through time (Figure 2.3a, Table 2.1), while total ER consistently increased with warming across all weeks (Figure 2.3b, Table 2.1). Concurrently, water-column chlorophyll-a concentrations decreased with increasing temperature, but total chlorophyll-a and the rate of decline with temperature varied through time (Figure 2.3c, Table 2.1). Temperature effects on chlorophyll-a were greatest in week 2, during the phytoplankton bloom.
Figure 2.3. (a) Total NPP, (b) total ER (fixed slope = 0.05), and (c) total water-column chlorophyll-a within mesocosms in all weeks at recent mesocosm temperatures (previous 7 days). Each point represents the total NPP, ER or chlorophyll-a concentration within one mesocosm at a weekly sampling event. Intensity of grayscale of points and lines increases with time, i.e. week 1 is the lightest gray, week 11 is the darkest gray.
Table 2.1. Linear mixed effects model comparison results for chlorophyll-a and oxygen flux through time. Models were ranked using AIC, and differences among ranked models were assessed using likelihood ratio tests. Significantly different models are indicated by \( p < 0.05 \), with the best model indicated in bold. First, models with different random effects structures were compared, that allowed either the intercept (1|week) or the intercept, slope characterizing the effect of temperature, and their covariance (temp|week) to vary among weeks. Then, for the top-ranked random effects structure, models with and without temperature as a fixed effect were compared to test whether temperature predicted variation in each response variable.

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2.3.2 Effects of warming and consumers on phytoplankton abundance and body size

By week 4, neither grazers nor warming had reduced phytoplankton abundance, as estimated by biovolume (Figure 2.4b). However, there was a marginally significant trend of decreased phytoplankton chlorophyll-a concentration across the temperature gradient, with no effect of
consumers (Figure 2.4a). There was no evidence of an interaction between grazers and temperature on phytoplankton abundance (Table 2.2).

Figure 2.4. (a) Phytoplankton chlorophyll-a (mg C per liter) with warming and consumer presence (gray circles represent consumer present mesocosms and black circles represent consumer absent mesocosms, n=10 and n=15, respectively). Solid line represents moderately significant temperature effect from linear regression analysis (p=0.055). (b) Phytoplankton biovolume, (log_{10} \text{um}^3 \text{per liter}), across the temperature gradient and in the presence (gray circles, n=10) and absence of consumers (black circles, n=15).

Despite the overall trend of increasing NPP with increasing temperature (Figure 2.3a), in week 4, total ecosystem productivity (NPP, oxygen flux) did not vary with temperature, but consumer presence significantly decreased total NPP across temperatures (Figure 2.5a, Table 2.2). Similarly, chlorophyll-a-specific NPP was invariant with temperature (Figure 2.5b, Table 2.2).
Warming and consumers had no effect on the median cell size (biovolume) of phytoplankton (Table 2.2), but shifts in size spectra indicate that the relative abundance of size classes was affected by consumer presence. Warming had no effect on the slope of the phytoplankton size spectrum in week 4 (Figure 2.6, Table 2.2). However, consumer presence steepened size spectrum slope from a mean of -0.61 (95% CI -0.68 to -0.55) in consumer-free mesocosms to -0.70 (95% CI -0.74 to -0.66) in mesocosms with consumers (Figure 2.6, Table 2.2), indicating that smaller phytoplankton were relatively more abundant than larger phytoplankton in consumer-present mesocosms compared to consumer-absent mesocosms. Further, consumers significantly decreased the intercept from 3.36 (95% CI 3.04 to 3.66) in mesocosms without consumers to 2.75 (95% CI 2.51 to 2.99) in mesocosms with consumers (Table 2.2), indicating that the relative abundance of large phytoplankton was lower in mesocosms with consumers than those without. There was no interaction between temperature and consumer presence on the phytoplankton size spectrum slope.
Figure 2.6. (a) Phytoplankton size spectrum linear regressions for consumer absent (gray solid lines) and consumer present mesocosms (warm-colored dashed lines). For consumer absent mesocosms, intensity of grayscale corresponds to intensity of warming. Similarly, in consumer present mesocosms, darker red colors represent warmer treatments and lighter colors represent cooler treatments. (b) Size spectrum slopes across temperature in mesocosms with consumers (gray circles, dashed line, n=10), and those without (black circles, solid line, n=15).
Table 2.2. ANCOVAs (type II sums of squares) of community responses in week 4 with temperature and consumer presence.

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During week 11, phytoplankton chlorophyll-a concentration declined with warming in the absence of consumers (Figure 2.7a, Table 2.3), and total phytoplankton biovolume similarly decreased with increasing temperature (Figure 2.7b, Table 2.3). However, due to growth of attached algae (see below), total algal biomass per mesocosm remained constant across the temperature gradient (Figure 2.8, Table 2.3).

Figure 2.7. Phytoplankton biomass estimated as (a) phytoplankton chlorophyll-a concentration (mg C per liter) and (b) phytoplankton biovolume (log_{10} um^3 per liter) with temperature in mesocosms without consumers in week 11 (n=15).
In week 11, temperature had no effect on total ecosystem productivity (NPP, oxygen flux) (Figure 2.9a, Table 2.3) or on total biomass-specific NPP (Figure 2.9b, Table 2.3).
Increased temperature significantly decreased median cell size (biovolume) of phytoplankton (Figure 2.10, Table 2.3), and further inspection of size frequency histograms suggests that warming increased the relative abundance of small phytoplankton. However, the slope and intercept of the phytoplankton size spectra were unaffected by warming in week 11 (Figure 2.11, Table 2.3). Thus, size spectra results suggest that warming did not affect the relative abundance of phytoplankton size classes, contrary to observed negative effects of temperature on median cell size.
Figure 2.10. Median phytoplankton cell biovolume (log_{10} um^3) across the temperature gradient in week 11 (n=15).
Figure 2.11. (a) Phytoplankton size spectra and (b) size spectra slopes for all mesocosms without consumers in week 11. Light gray lines represent low temperature mesocosms and dark gray lines represent high temperature mesocosms.

### 2.3.3 Effects of warming on algal resource availability

By the end of the experiment, the proportion of algae available as phytoplankton to herbivorous zooplankton had decreased with warming (Figure 2.13, Table 2.3), despite constant algal biomass across temperatures (Figure 2.8, Table 2.3). Meanwhile, the much less edible periphyton biomass growing on the sides of mesocosms increased (Figure 2.12a, Table 2.3) and benthic periphyton biomass remained constant across temperature treatments (Figure 2.12b, Table 2.3). One mesocosm was omitted from benthic, total biomass, and proportion analyses due to error in sampling benthic algal biomass within this mesocosm.
Figure 2.12. (a) Periphyton biomass (mg C) on the sides of mesocosms and (b) benthic algal biomass (mg C) within each mesocosm in week 11 (n=14).
Figure 2.13. Proportion of algal biomass as phytoplankton within mesocosms across the temperature gradient in week 11 (n=14).
Table 2.3. ANOVAs of community responses across temperatures in week 11.

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2.4 Discussion

Warming increased total ecosystem productivity of primary producers, as expected based on the temperature-dependence of metabolism and growth (Gillooly et al. 2001; Brown et al. 2004). Despite increased productivity at warmer temperatures, warming reduced the availability of algal resources to zooplankton herbivores. Warming decreased preferred resource availability to *Daphnia* by decreasing phytoplankton abundance and body size, and herbivory contributed to body size shifts across the temperature gradient. Interestingly, the effect of temperature on availability of algal resources was not proportional to the effect of temperature on ecosystem fluxes. Warming shifted dominant algal assemblages from phytoplankton to attached algae, driving the decline in food availability for filter-feeding consumers at high temperatures. Such shifts in plant resource availability have the potential to alter the rates of plant-herbivore interactions, with important consequences for trophic structure under warmer climates.

In the short term, warming shifted phytoplankton communities toward moderately lower chlorophyll-a concentrations. Despite this trend toward decreased chlorophyll-a with warming, total phytoplankton biovolume showed no response to temperature, suggesting that warming had no overall effect on total phytoplankton biomass. Decoupled trends in chlorophyll-a content and biovolume suggest that the temperature gradient affected growth conditions of phytoplankton in a way that altered cellular allocation of resources away from chloroplasts at higher temperatures (Felip and Catalan 2000). Though I did not document nutrient availability, I speculate that nutrient limitation following the bloom contributed to reductions in chlorophyll-a but not biovolume. Warming can intensify nutrient limitation via heightened organismal nutrient uptake (Gillooly et al. 2001; Allen and Gillooly 2009), and low phosphorus conditions have been shown to simultaneously decrease chlorophyll-a concentration and increase cell volume (Chen et al. 2011). Temperature may have also directly impacted species-specific chlorophyll-a to cell volume ratios (Rhee and Gotham 1981; Felip and Catalan 2000; Chen et al. 2011). Nevertheless, phytoplankton communities in week 4 maintained constant biovolume and productivity with warming despite evidence for decreased chlorophyll-a concentration within cells, suggesting higher photosynthetic rates per unit chlorophyll at warmer temperatures.
Consumers, but not warming, changed phytoplankton community structure via shifts in relative body size in week 4, despite constant total phytoplankton community biomass across temperatures. *Daphnia* increased the steepness of the phytoplankton size spectrum and decreased the intercept, indicating a decrease in the relative abundance of large phytoplankton with consumer presence. This suggests that *Daphnia* alter phytoplankton size structure via grazing preferentially on large size classes, a selective feeding behavior which is common among zooplankton (Katechakis et al. 2002; Sommer and Lewandowska 2011). The consumption of larger particles by *Daphnia* likely relieves competition for resources between large and small phytoplankton, allowing the less-preferred small particles to increase in relative abundance. Interestingly, warming had no effect on the size distribution of phytoplankton by week 4, contrary to previous research that has demonstrated temperature-induced increases in the steepness of phytoplankton size spectra (Yvon-Durocher et al. 2011). Temperature-induced shifts in phytoplankton body size, which are driven by either organismal-level physiological responses to temperature or community shifts toward smaller phytoplankton species (Atkinson 1994; Atkinson et al. 2003; Yvon-Durocher et al. 2011), may require longer timescales to be evident at the community-level. Further, decreases in phytoplankton body size with warming can be mediated by nutrient availability (Peter and Sommer 2013). In week 4, nutrient regimes across temperature may not have been different enough to generate observable differences in phytoplankton size spectra. Thus, under short timescales, consumers appear to be more important than warming in driving changes in phytoplankton size spectra.

The phytoplankton size spectra in week 4 do not support my hypothesis that warming alters the structure of primary producer assemblages, but consumer-effects on the size spectra support my hypothesis that phytoplankton community shifts are stronger in the presence of consumers. Further, the response of total phytoplankton biomass in week 4 rejects my hypothesis that phytoplankton abundance declines with warming and consumer presence. Notably, I saw no interaction between *Daphnia* presence and temperature on primary producer community structure, despite strong theoretical and experimental evidence that plant-herbivore interaction strength should increase with temperature (Vasseur and McCann 2005; O’Connor 2009; O’Connor et al. 2011; Gilbert et al. 2014). This may be explained by low *Daphnia* population abundance within mesocosms, with lowest abundance at high
temperatures. On average in week 4, *Daphnia* population size was less than one individual per liter. In experimental settings, *Daphnia* densities of 6.2 individuals per liter are low enough to release grazing pressure and allow recovery of edible phytoplankton species (Kerfoot et al. 1988). *Daphnia* are sensitive to temperature increases and exhibit population declines in natural systems with warming (Steiner 2004; Winder and Schindler 2004; Dupuis and Hann 2009). Therefore, it is possible that consumer-driven decreases in phytoplankton abundance and increased plant-herbivore interaction strength would not be detectable at the community or ecosystem level in this system due to the thermal niche of *Daphnia pulex*.

In the absence of cladoceran grazers, total algal biomass was unaffected by temperature at longer timescales. However, there was a significant shift toward dominance by periphyton and benthic algae by week 11, with a decrease in the absolute and relative availability of pelagic algal resources with warming. This supports my hypothesis that direct effects of warming decrease the relative abundance of available algal functional groups to zooplankton consumers. Importantly, biomass estimates and the proportion of available resources declined continuously across the temperature gradient, rather than exhibiting a sudden decrease at a particular thermal limit. This suggests that the temperature gradient within this study did not push algal assemblages over their physiological thermal maxima, but rather tracked steady non-lethal responses to warming.

Within the literature, the direct effect of temperature on standing primary producer biomass is rarely investigated in the absence of consumers. Studies have found that warming produces inverted pyramids in pelagic aquatic systems, with no change in zooplankton biomass with warming, but significant decreases in phytoplankton biomass (O’Connor et al. 2009; Yvon-Durocher et al. 2011). Shurin and colleagues (2012) investigated the effects of warming on an entire pond food web, and also found a top-heavy trophic structure, with decreases in both pelagic and benthic primary producers. My results conflict with those of Shurin *et al.* (2012), as I saw no evidence for negative temperature effects on benthic algae. This is likely explained by the lack of benthic consumers within mesocosms; periphyton biomass has been shown to increase with temperature in the absence of consumers, but significantly decrease with temperature in the presence of consumers (Cao et al. 2014). Importantly, mesocosms were likely nutrient limited by week 11, and increasingly limited in high temperature
treatments, contributing to biomass shifts among algal groups. Macroalgae generally outcompete phytoplankton for resources, leading to lower phytoplankton biomass with increased macroalgal abundance (Fong et al. 1993; Blumenshine et al. 1997). Thus, if periphytic algae have an advantage in both their physiological response to temperature and their competitive ability to uptake nutrients over phytoplankton, we would expect the observed shift toward periphyton-dominated systems.

Despite strong declines in phytoplankton cell size with warming, I saw no effects of temperature on planktonic size spectra in week 11. At this time point, warming may have shifted the shape of the phytoplankton size distribution in such a way that linear analysis of the size spectrum could not accurately capture size shifts within phytoplankton community structure. Closer inspection of the frequency distributions of organism sizes suggests that smaller cells were relatively more abundant at warmer temperatures. Thus, my results support previous findings that warming shifts phytoplankton communities toward assemblages comprised of smaller individuals with lower standing biomass (Yvon-Durocher et al. 2011). The response to temperature of cell size in week 11 may reflect direct physiological temperature-effects, or be driven by temperature-dependent nutrient limitation, which can select for smaller phytoplankton (Winder and Schindler 2004; Peter and Sommer 2013). Smaller cell size offers a competitive advantage under low nutrients by requiring less nutrient uptake per capita, as predicted by the direct scaling of metabolic rate and nutrient uptake with temperature and body size (Gillooly et al. 2001; Allen and Gillooly 2009). Following the observed consumer-preference for large phytoplankton in week 4, these consistent warming-induced declines in phytoplankton body size further reduce preferred resource availability with the potential to change the ways in which consumers and resources interact.

In both the long and short term, the availability of plant resources to filter-feeding zooplankton decreased across the temperature gradient in my experiment. In the short term, this decrease in food availability was driven by consumers, which decreased the relative abundance of preferred food types via size-selective grazing. Although this study did not include multi-species consumer assemblages, in natural systems, temperature- and grazer-induced declines in available food could alter competitive dynamics among consumers. Novel competitive interactions may arise among size-selective zooplankton that previously
experienced some refuge within their feeding niche (DeMott 1982; Narwani and Mazumder 2010). These interactions, in combination with species-specific thermal tolerances, could shift the dominant grazers within the system, with implications for ecosystem-level effects (Steiner 2004; Dupuis and Hann 2009). Over longer timescales, warming may destabilize consumer-resource dynamics as zooplankton overexploit their already diminished food sources, leading to a crash in herbivore populations (Beisner et al. 1997).

I have demonstrated that the community structure of freshwater primary producer assemblages varies across a temperature gradient, with shifts in dominant algal functional groups with warming. My results suggest that an important consequence of environmental warming could be a reduction in resource availability to herbivorous zooplankton, with a shift toward benthic-dominated systems. The effects of warming on the availability of resources may have predictable broad-scale consequences for the structure of aquatic systems, but so far appear to cause trends in biomass or abundance with warming to deviate from first-order predictions based on extensions of metabolic scaling with temperature. These responses should be incorporated into consumer-resource models and community theory to more accurately predict the rates and stability of trophic dynamics with warming.
Chapter 3

Conclusion

3.1 Summary

Warming shifted freshwater algal assemblages toward lower phytoplankton community biomass, higher relative abundance of small body sizes, and decreased availability of preferred resource types to herbivorous zooplankton like *Daphnia*. In the short term, *Daphnia* were more important than warming in driving preferred resource limitation. Under longer timescales in the absence of consumers, algal assemblages shifted from pelagic to attached species with warming, with lower phytoplankton biomass but constant total algal biomass across temperatures. Thus, both direct effects of warming and selective consumption by consumers constrain preferred resource availability, with the potential to feedback into consumer-resource dynamics and food web structure.

3.2 Implications for trophic dynamics and community structure

*Daphnia*-phytoplankton population dynamics exhibit a classic predator-prey boom-bust cycle directly following the spring phytoplankton bloom, with subsequent increasingly stable population dynamics (Lampert et al. 1986). This eventual stability is partially driven by *Daphnia*’s own exploitation of preferred resources, which increases the abundance of inedible phytoplankton species, thus maintaining stable phytoplankton biomass and high diversity (McCauley and Briand 1979; Kerfoot et al. 1988). My findings align with this research, which shows that *Daphnia* limit the availability of their preferred resources. Further, I’ve found that warming exacerbates changes in food availability in the same direction as consumption by *Daphnia*. As a result, warming may lead to local extinctions of *Daphnia* populations due to high thermal stress or the inability to maintain population growth due to limited resources (Beisner et al. 1997). Even if *Daphnia* populations persist with warming due to non-lethal temperature increases or thermal adaptation, their equilibrium biomass will likely be lower than under cooler-temperature conditions. Presumably, this decreased carrying capacity would
be driven by feedbacks between increased consumption and decreased phytoplankton biomass, with insufficient edible resources to sustain large population numbers.

Although *Daphnia* are considered to be the dominant competitors for phytoplankton within freshwater systems, their temperature sensitivity and observed preference for large particles may shift competitive hierarchies in favor of smaller, more temperature tolerant zooplankton. Indeed, competitive outcomes between small zooplankton species and *Daphnia* are highly variable and often dependent on the availability and size structure of algal resources, with smaller species being more effective competitors at low food concentration (Neill 1975; DeMott 1982; Romanovsky and Feniova 1985). In the field, warmer temperatures have been associated with shifts from *Daphnia*-dominated systems to herbivore assemblages of smaller-bodied zooplankton (Steiner 2004; Dupuis and Hann 2009). These smaller-bodied taxa may also benefit from the observed increase in the relative abundance of smaller phytoplankton particles. Further, changes in the size structure and diversity of zooplankton communities have important implications for the foraging efficiency and food quality of higher-level consumers.

Shifts within benthic community structure with warming occur simultaneously with shifts in pelagic systems (Dossena et al. 2012; Shurin et al. 2012). I found a positive temperature effect on periphyton growing on the sides of mesocosms, and no temperature effect on benthic algal assemblages. Thus, herbivores which feed on attached algae will be faced with less severe resource limitation with warming compared to zooplankton, since direct temperature effects do not decrease the abundance of attached algae. However, benthic herbivores must still cope with the direct physiological effects of thermal stress, and it is likely that increased consumption rates with warming will decrease attached algal biomass (Hillebrand 2009; Shurin et al. 2012; Cao et al. 2014). My research offers limited predictions for the structure of benthic communities with warming in natural systems, since I investigated attached algae exclusively in the absence of higher trophic levels. Nevertheless, my findings offer insight to direct temperature effects on attached algal biomass.

The observed temperature shifts within pelagic and benthic algal communities have important implications for higher trophic levels and resulting food web structure in aquatic systems. Understanding how the effects of temperature alter trophic interactions is vital to predict the response of communities and ecosystems to a changing climate.
3.3 Recommendations for future research

While several potential mechanisms for how temperature alters plant-herbivore interactions are elucidated in the current work, additional research should be done within this field to further understand the direct and indirect effects of temperature on consumer-resource interactions. In particular, empirical tests that monitor temperature effects on *Daphnia* and phytoplankton population cycles at high resolution are necessary. Such tests could help to disentangle potential temperature-induced feedbacks between herbivore and primary producer populations and allow us to incorporate the indirect effects of temperature into consumer-resource models. It is likely that nutrient limitation plays a large role in determining community structure of primary producers across temperature, so future experimental work should strive to maintain constant nutrient levels and perform frequent nutrient sampling in order to isolate the effects of temperature.

Subsequent research should also consider higher trophic levels. Increased consumption of herbivores by predators with warming can have drastic effects on herbivore populations and whole community level responses (Beisner et al. 1997), and size-selective consumption of *Daphnia* by fish is already known to alter community structure in lakes and generate trophic cascades (Brooks and Dodson 1965). This work would help us to incorporate trophic dynamics into the theoretical framework provided by metabolic theory.
Bibliography


