NUTRIENTS IN A CHANGING WORLD: THE EFFECTS OF WARMING AND PREDATOR PRESENCE ON PREY QUALITY

by

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Abstract

The field of climate change impacts on ecology primarily focuses on the measures of abundance and distribution of individuals to assess organism response, but the measure of organism quality is not often applied to assessing organism response within food webs. The abiotic factor of temperature has known effects on organism quality, but it is unclear how the biotic factor of predator presence impacts organism quality. The goal of my thesis was to investigate (a) the combined effects of warming and predator presence on prey nutrient quality, and (b) how temperature affects organism quality and the nutritional needs of consumers. To address the combined effects of warming and predator presence on one measure of prey quality - organism body size - I conducted a meta-analysis on 14 papers that tested both warming and predator presence on prey body size in aquatic systems. Across all studies, I found no net effect of warming on body size, a large increase in prey body size with predator presence, and an additive effect of the two factors combined. I then conducted a laboratory experiment using the primary producer *Scenedesmus obliquus*, the primary consumer *Daphnia pulex*, and the secondary consumer *Chaoborus americanus* to investigate temperature mediated changes in algal quality and consumer nutritional needs (measured using the fatty acid profile of algae that affected *D. pulex* population size and *C. americanus* growth rates). Overall, we observed changes in *S. obliquus* quality with temperature and mild cascading effects of these changes on *D. pulex* and *C. americanus.* Further investigation is needed into the effects of warming and predator presence on other nutrients (such as carbohydrates and proteins that may respond differently to temperature), and if the relationship between body size and quality holds true for all organisms. Overall, my thesis provides insight into how predator presence can have a stronger effect on organism body size than warming and suggests greater care must be taken when interpreting the

iii

results of studies that assess the effects of temperature on organism body size in the absence of

biotic factors.

Lay Summary

Both temperature and predator presence affect important aspects of organism biology such as reproduction and growth. As temperature increases due to global warming, it remains unclear as to whether greater focus should be given to the effects of temperature over other factors such as predator presence. Specifically, climate effects on organism quality have not been well studied within food webs. Temperature and predator presence can affect organism quality but less is known about their combined effects. The goal of this study was to understand (1) how temperature and predator presence compare for their effects on organism quality, and (2) how temperature effects organism quality. Through a review of the current literature, we found predator presence increases prey body size (correlated with quality) and has a much stronger effect on prey body size than temperature. Through a lab experiment we found warming decreases organism quality and the nutritional needs of consumers.

Preface

Chapter 2 of this dissertation is original, unpublished, and independent work by author, C. M. Di Filippo. The work on project ideas, methodology, data analysis and writing were conducted under the supervision of Dr. Michelle Tseng.

Chapter 3 of this dissertation is a collaboration with members of the Tseng Lab (i.e., supervisor Dr. Michelle Tseng, undergraduate Madeline Fung, and researcher Dr. Ian Forster). Some of the data in Chapter 2 is included in a related publication by M. Tseng, C. M. Di Filippo*, M. Fung*, J.O. Kim*, I.P. Forster, Y. Zhou (with " * " indicating equal contribution). I was responsible for helping to count zooplankton populations, measure insect larvae growth rates, and feed and maintain insect larvae populations. I was not involved in the growing or quantifying of algae.

Table of Contents

List of Tables

List of Figures

List of Equations

List of Abbreviations

Abbreviations and definitions are based on those presented in Twining et al. (2016).

Fatty acid – nomenclature

Fatty acid abbreviation	Fatty acid name	Fatty acid Nomenclature	Saturation type	Omega type (3 or 6)
ALA	α-Linolenic acid	18:3n-3	Polyunsaturated	Omega-3
DHA	Docosahexaenoic	22:6n-3	Polyunsaturated	Omega-3
EPA	Eicosapentaenoic	20:5n-3	Polyunsaturated	Omega-3
LIN	Linoleic acid	18:2n-6c	Polyunsaturated	Omega-6
Oleic	Oleic acid	18:1n-9c	Monounsaturated	Omega-9
ARA	Arachidonic acid	20:4n-6	Polyunsaturated	Omega-6

Note: c = cis configuration of fatty acid chain

Example of fatty acid nomenclature:
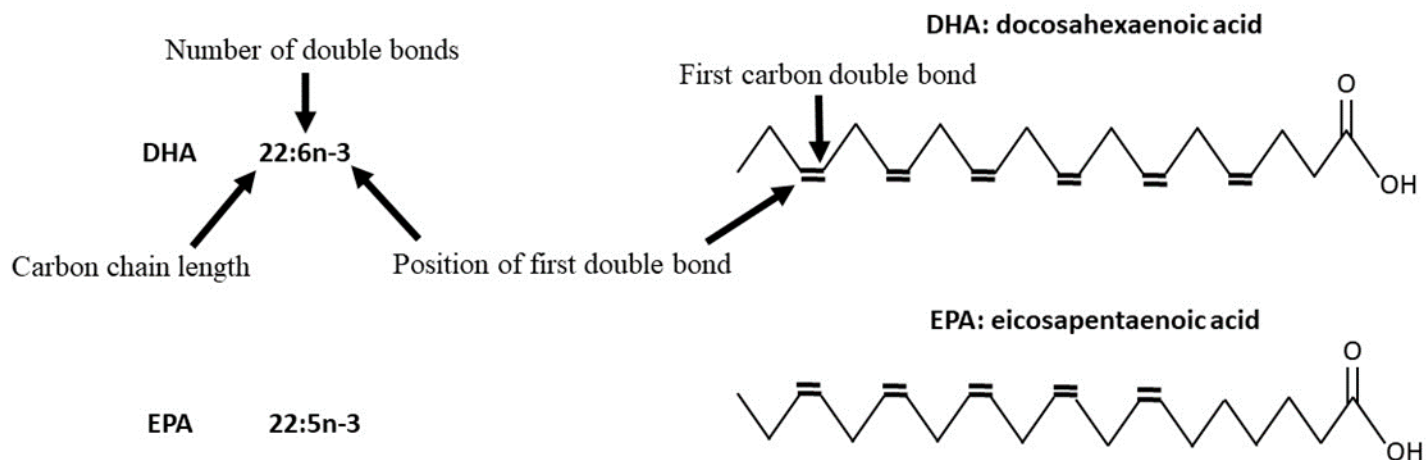
Fatty acid – Number of double bonds

Number of bonds abbreviation	Saturation name	definition
SFAs	Saturated fatty acids	Fatty acids with the hydrocarbon chain including single bonds between carbon atoms.

UFAs	Unsaturated fatty acids	Fatty acids with the hydrocarbon chain including a minimum of one double bond between carbon atoms.
MUFAs	Monounsaturated fatty acids	Fatty acids with the hydrocarbon chain including one double bond between carbon atoms.
PUFAs	Polyunsaturated fatty acids	Fatty acids with the hydrocarbon chain including more than one double bond between carbon atoms.
HUFAs	Highly unsaturated fatty acids	Polyunsaturated fatty acids with the hydrocarbon chain including more than two double bonds between carbon atoms.

Fatty acid – chain length

Carbon chain length abbreviation	Carbon chain length	Definition
LCFAs	Long-chain fatty acids	Fatty acids containing greater than twenty carbon atoms.
LC-PUFAs	Long-chain polyunsaturated fatty acids	Fatty acids containing greater than twenty carbon atoms and the hydrocarbon chain including more than one double bond between carbon atoms.

Fatty acid – position of first double bond

Fatty acid abbreviation	Fatty acid name	Definition
Omega-3	Omega-3 polyunsaturated fatty acids	Fatty acids with the hydrocarbon chain involving the first double bond on the third carbon atom from the methyl end.
Omega-6	Omega-6 polyunsaturated fatty acids	Fatty acids with the hydrocarbon chain involving the first double bond on the sixth carbon atom from the methyl end.

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I am deeply grateful for the constant support and intellectual freedom provided by my supervisor, Dr. Michelle Tseng. Her dedication to mentorship provided strong growth in my skills as a researcher and scientist.

I thank my committee members, Dr. Mary O'Connor, Dr. Brian Hunt, and Dr. Katie Marshall for their professional insight, advice, and guidance, especially during the tougher times of thesis planning due to unprecedented times.

I thank Madeline Fung for her bright energy and thoughtful remarks on designing and executing our laboratory experiment.

I thank Dr. Ian Forster for his insight on fatty acid analysis and help with analyzing our fatty acid samples.

I am grateful for the Tseng lab that is ever growing with kind-hearted and vibrant personalities that provided laughs and support during my degree.

I am grateful for the opportunity to work with the exceptional faculty, staff, and fellow students at UBC who never cease to inspire me on my next adventure.

Special thanks are owed to my family, whose willingness to let me grow, explore, and play has allowed me to pursue my passions. Their constant love and support are forever acknowledged.

Dedication

This dissertation is dedicated to my beloved canine companions, Charles and Toby.

You loved me whether I accomplished all or nothing. I miss you both every day.

Chapter 1: Introduction

1.1   Effects of climate warming on organisms

Climate change is expected to shift the distribution, intensity, and timing of abiotic factors that have known effects on organism distribution and abundance (Bale et al., 2002; Elith and Leathwick, 2009; Harley et al., 2006). For example, warming is predicted to affect distributions of terrestrial species, with species distributions shifting to higher latitudes and elevations in response to warming (Chen et al., 2011). Additionally, the abundance of herbivorous insects is expected to change as a result of altered growing seasons and plant abundance caused by warming (Bale et al., 2002; Goulson, 2019). Ecological studies of the effects of climate change often focus on quantifying changes in abundance and distribution of species, but these two responses may not be the only important measures of the effects of warming. Individual characteristics such as organism quality have important impacts on organism and consumer health but have not been well studied in the context of climate change.

1.2   Defining organism quality

All organisms are comprised of the same four major classes of macromolecules: lipids, carbohydrates, proteins, and nucleic acids (Hessen, 2008). These macromolecules are primarily made up of the elements oxygen (O), hydrogen (H), carbon (C), and nitrogen (N). We define organism quality by the composition of these elements within a macromolecule, and the proportion of macromolecular classes within organisms. For example, all four macromolecular classes have high quantities of O, H, C, and N; lipids are comprised of a large number of C and H, carbohydrates contain high quantities of C and O, proteins are rich in N, and nucleic acids are

1

rich in P (Hessen, 2008). Increases in C, N, and P within organisms can lead to increased quantities of lipids, carbohydrates and proteins and all three macromolecules contribute to increased growth rates (Hessen et al., 2007).

1.3   Addressing changes in organism quality through changes in fatty acids

Fatty acids are important for proper growth and development (Spector and Kim, 2015). In general, deficient quantities or improper ratios of fatty acids can lead to decreased growth rates and smaller body size in many organisms (Parrish, 2009; Simopoulos, 1999). The necessity of fatty acids for proper development has resulted in the measurements of fatty acid quantity and composition being used to assess organism quality. Not all fatty acids are synthesized de novo (i.e., essential fatty acids) and these must therefore be consumed through a food source (Spector and Kim, 2015). Changes in temperature have documented effects on fatty acid quantities and ratios, with warming causing, e.g., decreased quantities of essential omega-3 polyunsaturated fatty acids in phytoplankton (Guschina and Harwood, 2009; Hixson and Arts, 2016). A decrease in omega-3 fatty acids in phytoplankton has the potential to negatively affect consumer health, due to reductions in zooplankton fecundity and population size (Navarro et al., 2019).

1.4   Effects of temperature and predator presence on organism quality

Temperature is a key abiotic factor and predator presence a key biotic factor that can potentially alter organism quality (Fuschino et al., 2011; Pessarrodona et al., 2019). While the effects of temperature on organism quality are generally well-understood (Fuschino et al., 2011; Laws and Joern, 2013; Schlechtriem et al., 2006), the effects of predator presence on organism quality are not well understood. Consumptive and non-consumptive predator effects may impact

organism quality via their effects on prey body size because body size is often correlated with nutrient weight (Arendt, 2007; Breck, 2014; Gorokhova, 2019). Organism body size is also strongly affected by temperature (Atkinson, 1994; Daufresne et al., 2009; Kingsolver and Huey, 2008), thus, we can use body size to compare the effects of warming and predator presence on organism quality.

1.5   Structure and objectives

The objective of this study is to investigate the effects of warming and predator presence on organism quality. Specifically, we aim to understand how temperature affects diet quality and the nutritional needs of consumers, and how the combination of factors, such as temperature and predator presence, affect organism quality. This thesis consists of four chapters: an introduction, data chapter one, data chapter two, and conclusion. The first data chapter assessed the effects of warming and predator presence on prey quality (as defined by body size) using a factorial meta-analysis. I compiled studies that tested both predator presence and absence with warm and cold treatments for their effects on prey body size. The second data chapter assessed the effects of temperature on diet quality and on the nutritional needs of consumers using a laboratory experiment. Algae (*Scenedesmus obliquus*), zooplankton (*Daphnia pulex*), and insect larvae (*Chaoborus americanus*) were reared at three different temperatures and each trophic level was fed to the subsequent tier (i.e., algae to *D. pulex* and *D. pulex* to *C. americanus*). This thesis will contribute to our understanding of how predator presence and temperature impact organism quality and which factor may be of higher importance for their effects on quality with ongoing climate change.

Chapter 2: Meta-analysis on the effects of predator presence and warming on prey nutrient quality

2.1 Introduction

In this chapter I use a meta-analysis to test for the general effects of warming and predator presence on prey body size. Below I describe the rationale behind using body size as a proxy for organism quality, and I summarize the known independent effects of warming and predator presence on prey body size.

2.1.1 The relation between body size and organism quality

Body size has been a well-documented measure of organism quality, as nutrient weight is directly correlated with organism body size (Ali et al., 2001; Arendt, 2007; Breck, 2014; Cui and Wootton, 1988; Ghomi et al., 2014; Gorokhova, 2019; Ho et al., 2020). For example, Ghomi et al. (2014) found a positive correlation between goby body length and the weight of three essential amino acids per mg of dried fish tissue (μmol/mg). They concluded that larger individuals of this species had higher essential amino acid content per mg of dried fish tissue than smaller individuals (Ghomi et al., 2014). Additionally, within species of zooplankton, individual zooplankton with higher body weight have increased lipid storage capacity (i.e., an increase in neutral to polar lipid ratios, used as proxy for mass-normalized lipid storage) (Gorokhova, 2019). The authors concluded that zooplankton of higher weight were likely of higher nutritive value to predators (Gorokhova, 2019).

2.1.2 The effects of temperature on organism body size

Temperature can have strong effects on organism body size (Angilletta et al., 2004; Atkinson, 1994; Atkinson and Sibly, 1997; Bergmann, 1847; Blanckenhorn, 2000; Daufresne et al., 2009; Gutierrez-Pinto et al., 2014; James, 1970; Kingsolver and Huey, 2008; Sheridan and Bickford, 2011). Three general patterns between temperature and body size have been described. Bergmann's rule describes the pattern observed across geographic regions in which small-sized species tend to inhabit warmer regions (Bergmann, 1847). James's rule describes the pattern that, within a species, a warmer environment generally results in populations with smaller body size (James, 1970). Finally, the temperature-size rule (TSR) describes the pattern whereby individuals grown at warmer temperatures tend to mature at smaller adult sizes, compared to individuals grown at colder temperatures (Atkinson, 1994). Warming could result in smaller individuals if individual development rates increase at higher temperatures, leading to shortened development time and thus less time for organisms to gain biomass (Arendt, 2011; Frouz et al., 2002). Organisms could also be smaller at warmer temperatures if they allocate resources to functioning under heat stress, rather than to growth (Angilletta et al., 2004; Atkinson, 1994; Atkinson and Sibly, 1997; Blanckenhorn, 2000). Although there is considerable intra- and interspecific variation in the effects of warming on body size it is commonly observed that warming generally decreases organism body size (Cui and Wootton, 1988; Forster et al., 2012; Sotoyama et al., 2018). At the community level, warming can also lead to shifts from larger-bodied species to smaller-bodied species through size-selective predation removing larger-bodied individuals from prey populations (MacLennan et al., 2015). Given the commonly reported link between organism body size and nutritive quality (especially in aquatic organisms), ongoing climate warming could

significantly decrease the availability of nutrients in ecological communities, via decreases in organism body size.

2.1.3 The effects of predator presence on prey body size

Predator presence can also significantly affect organism body size. Predator presence can influence body size of prey through consumptive and non-consumptive effects (Brooks and Dodson, 1965; Calder, 1984; Cohen et al., 1993; Dodson, 1989; Hart and Bychek, 2011; Lima and Dill, 1990; Ludwig and Rowe, 1990; Paine, 1976; Peters, 1983; Scharf et al., 2000; Schmitz et al., 2010). Consumptive predation occurs when predators actively remove prey from the population (Pessarrodona et al., 2019). Non-consumptive predator effects do not directly remove prey from the population but rather influences prey behaviour through predator presence or predator cues (Hill and Weissburg, 2013).

Consumptive effects of predators can affect prey body size via size-selective predation (Pessarrodona et al., 2019). With size-selective predation, predators preferentially prey on a certain size class and remove them from the prey population. For example, in aquatic ecosystems, tactile predators (i.e., invertebrate predators detecting prey using hydrodynamic disturbances caused by movement) tend to select prey that are smaller and easier to handle. Conversely, visual predators (e.g., fish) are better able to see larger prey items, and thus tend to remove larger-sized prey from the population (Brooks and Dodson, 1965; Hart and Bychek, 2011). With respect to non-consumptive effects of predators, predator presence can alter prey size if prey spend less time foraging and more time hiding (Davenport et al., 2020; Lima and Dill, 1990; Ludwig and Rowe, 1990). The reduced foraging time can result in decreased nutrient

intake and reduced energy for individual growth and reproduction (Lima and Dill, 1990; Ludwig and Rowe, 1990).

Although there is variation in the overall effects of predator presence on prey body size, it has been widely suggested that predation generally decreases prey body size (Atkinson, 1994; Calder, 1984; Pessarrodona et al., 2019). However, notable exceptions to this pattern include size escapes, such as the effects of sea star predation on mussels (Paine, 1976). Mussels that were too large to be consumed by predators persisted and created a mussel population of larger-sized individuals (Paine, 1976).

There is also a large body of literature that has investigated how predator presence affects the evolution of prey life-history strategies (Atkinson, 1994; Calder, 1984). Predator-mediated selection by consumption can result in the evolution of smaller or larger prey size at maturity (Atkinson, 1994; Calder, 1984). Whether body size shifts are due to ecological (e.g., consumptive/non-consumptive effects) vs evolutionary (or both) effects affects long-term predictions on how warming and predator presence may affect prey body size, however distinguishing between these mechanisms is often difficult. In the studies I include in the meta-analysis, these mechanisms were not often discussed and as a result, were not included in my analyses.

2.1.4   The combined effects of warming and predator presence on organism body size

Both warming and predator presence often lead to smaller-sized prey (Angilletta et al., 2004; Atkinson, 1994, 1994; Atkinson and Sibly, 1997; Blanckenhorn, 2000; Calder, 1984). Additionally, because warming can increase predator consumption rates, some studies predict that warming should accentuate the effects of predators on prey body size (Atkinson, 1994).

Whether the combined effects of warming and predator presence are greater or less than the sum of their main effects is unknown, and I address this knowledge gap in this chapter. Assuming that body size is positively correlated with organism quality (Ali et al., 2001; Arendt, 2007; Breck, 2014; Cui and Wootton, 1988; Ghomi et al., 2014; Gorokhova, 2019; Ho et al., 2020), investigating the combined effects of these two common factors (warming, predator presence) across multiple studies and taxonomic groups, can significantly improve our understanding of how ongoing climate warming may affect nutrient availability in predator-prey communities.

2.1.5 Study objectives

The aim of my study was to use a meta-analysis to test the hypotheses that, (1) temperature and predator presence affect prey body size, and (2) temperature and predator presence differ in their effects on prey body size. I compiled 18 potential studies for the meta-analysis, and four of these were excluded due to missing values or inappropriate data. Although the search criteria were open to both aquatic and terrestrial studies, the 14 studies included here investigated only aquatic predators and prey. To test hypotheses (1) and (2), I conducted a factorial meta-analysis with Hedge's d as the effect size metric and assessed whether Hedge's d for temperature differed from that for predator presence. Additionally, I tested whether the combined effects of temperature and predator presence were additive, synergistic, or antagonistic (definitions below). I also tested whether Hedge's d differed among two predator types (tactile vs. visual) and among two predator modes (consumptive vs. non-consumptive) to help explain the effects of temperature and predator presence on prey body size.

2.2 Predictions

Warming, predator presence, and their interaction

I predict that warming will reduce prey body size (Angilletta et al., 2004; Atkinson, 1994; Atkinson and Sibly, 1997; Blanckenhorn, 2000) (Box 2-1). Additionally, I expect the effect of warming on body size to be weaker than the effects of predator presence, and weaker than the effects of both warming and predator presence combined. This prediction is based on results from several studies showing a stronger effect of predator presence versus warming on prey life history traits (including body size) (Brooks and Dodson, 1965; Hart and Bychek, 2011; Tseng and O'Connor, 2015).

The combined effect of predator presence and warming is unknown; however, three possible outcomes are that the two factors have additive, synergistic, or antagonistic effects on body size.

Box 2-1 Predicted effects of warming, predator presence, and the interaction between warming and predator presence on prey body. The possible outcomes for the interaction effect sizes are described in detail in the Methods, Section: Interpretation of the interaction effect size.
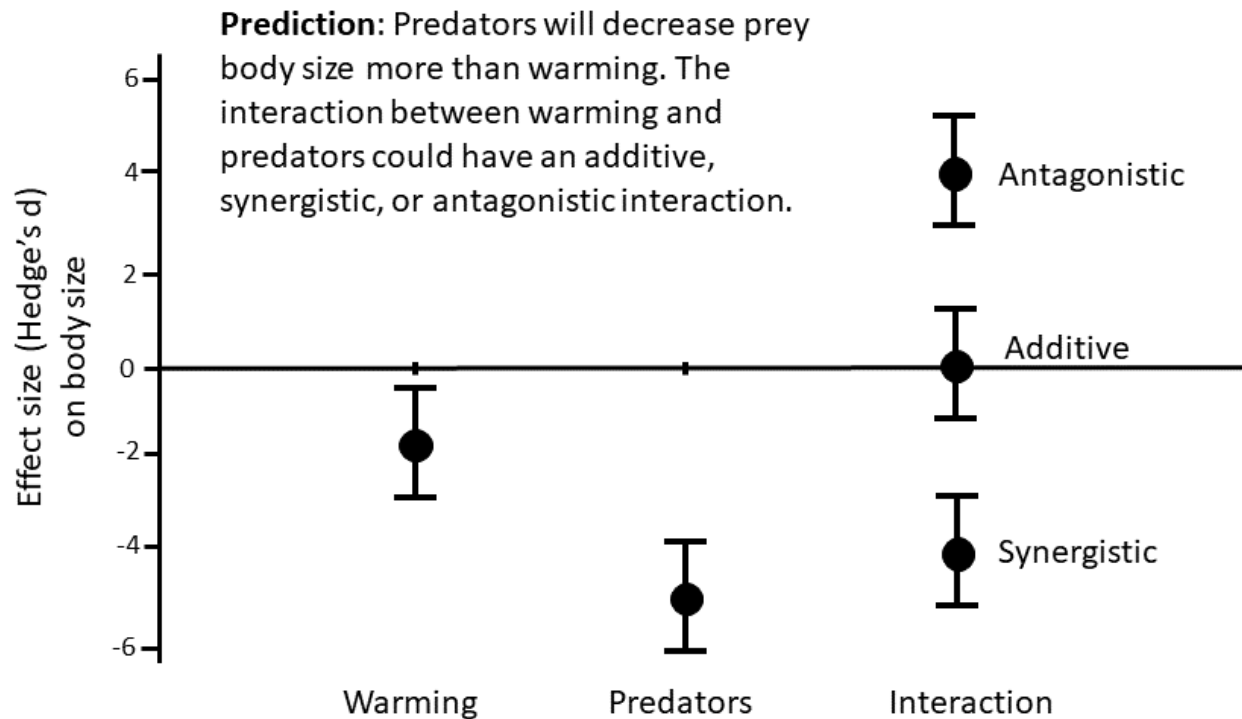
Effects of predation effect and predator foraging mode

I predict that consumptive effects of predators will have a larger effect on prey body size compared to non-consumptive effects of predators (Davenport et al., 2020; Pessarrodona et al., 2019) (Box 2-2). The direction of the effect may depend on whether the predator searches for prey using visual or tactile cues (Brooks and Dodson, 1965; Hart and Bychek, 2011). Visual predation should decrease prey body size and tactile predators should increase prey body size. I do not have a priori reason to expect that the magnitude of the effect size of visual predators will differ significantly from that of tactile predators.

Box 2-2 Potential combined effects of warming and predator presence on prey body size with predator presence grouped by foraging mode and predation effect.

Prediction: Visual predators will decrease prey body size while tactile predators will increase prey body size. Consumptive predation will decrease prey body size the more than non-consumptive predation.
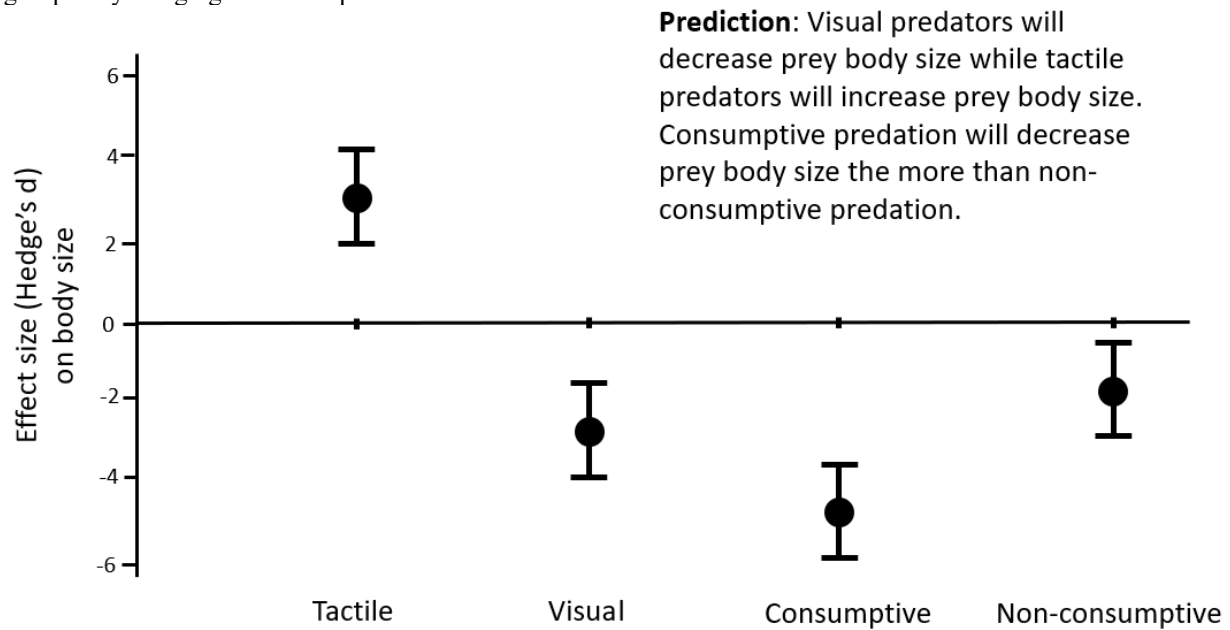
2.3   Methods

2.3.1   Data collection

I used Web of Science to find studies that examined the combined effects of warming and predator presence on prey body size. With help from Dr. Tseng, I conducted four searches: 1) Search terms: (("temperature" or "warm*" or "heat near/0 stress") and ("predation" or "predator near/0 presen*" or "predator near/0 absen*") near/10 "body size") (n = 85 papers); 2) all papers that cited "Riessen 1999" (n = >100 papers); 3) Search terms ("predat*" and "temperature" and "body" and "experiment") (n = 371 papers); 4) Search terms (("pred*" and "temperature"), refine (search within): 'body size') (n = 42 papers). We also found relevant papers in the references section of the above papers.

Studies were included in the meta-analysis if they included a measure of organism body size (length or mass) and if they included a predator treatment (presence or absence of consumptive or non-consumptive predator effects), a temperature treatment (minimum two temperatures), and a combined treatment with both predator presence and warming. For papers that tested a range of temperatures, I used the coldest temperature tested as the 'cold' treatment, and the warmest temperature tested as the 'warm' treatment. If a range of temperatures was included for any of cold or warm temperature treatments, I used the body size associated with the average cold and average warm temperatures. When papers presented body size measures in figures, I used the Figure Calibration plugin (Hessman, 2009) of Image J (Schindelin et al., 2012) to digitize data.

For each paper I recorded body size, temperature, predator presence, and if temperature fluctuations were present. For each prey species I recorded the genus, species, whether it was aquatic or terrestrial, and the life history stage. For each predator species I recorded similar

variables with the additional information of whether the predator mode was consumptive or non-consumptive, and whether the predator exhibited visual or tactile predation behaviour. I also recorded potential explanatory variables that could affect prey body size, but these variables were not included as covariates in my analysis due to there being no way to include covariates for the specific analyses conducted.

Because of the small number of studies included in the meta-analysis, the findings of my study may be more exploratory in nature (power analysis presented below). I included papers that tested the effects of warming and predator presence, and warming and predator presence with the additional factors of diet variation or competitor presence (Field studies with uncontrolled diet or competition = 3 studies, and laboratory studies with direct testing of diet or competition = 4 studies; see Table 2-1 for list of studies). To estimate effect sizes, I took the average body size from diet or competitor treatments to have a single effect size value for these papers. The inclusion of papers with these additional factors may increase the variance within predator and temperature categories, as diet type and competitor species can impact growth and body size through reduced resource quality or availability (Pavón-Meza et al., 2007; Peckarsky et al., 2001).

Table 2-1 Original 18 papers that had treatments for warming, predator presence, and warming plus predator presence. Papers highlighted in grey were excluded based on missing data or community measurements of body size. I included papers that may have potential diet or competitor effects with the understanding that they may contribute to variation in body size.

Article	Missing data for effect size calculations (Y/N)	Measured community body size (Y/N)	Uncontrolled diet or competitor effects (field experiments) (Y/N)	Direct testing of diet or competitor effects (Y/N)
Havens et al., 2015	**Y**	**Y**	**Y**	N
Cavalheri et al., 2019	N	N	N	N
Costa and Kishida, 2015	N	N	N	N
Gilbert, 2011	N	N	N	**Y**
Grigaltchik et al., 2016	N	N	N	**Y**
Guerra et al., 2012	**Y**	N	N	N
Huss et al., 2010	N	N	**Y**	N
MacLennan et al., 2015	N	**Y**	**Y**	N
MacLennan and Vinebrooke, 2016	N	N	N	**Y**
Pavón-Meza et al., 2007	N	N	N	N
Peckarsky et al., 2001	N	N	**Y**	N
Scherer et al., 2013	N	N	N	N
Tseng et al., 2019	**Y**	N	N	N
Tseng et al., 2018	N	N	N	N
Tseng and O'Connor, 2015	N	N	N	N
Weetman and Atkinson, 2002	N	N	N	**Y**
Weetman and Atkinson, 2004	N	N	N	N
Zhao et al., 2014	N	N	**Y**	N

2.3.2 Effects of predator presence and temperature on body size

To compare the effects of predator presence and temperature on prey body size across studies, I used Hedge's d as the standardized mean difference (SMD) for each study. I chose a SMD over an odds ratio (OR) or correlation coefficient (r) because the studies of interest compare means of a numerical response variable between two or more groups, rather than measure the success or failure between group treatments (ie. OR) or the comparison of the relationship between two numerical variables (i.e., r) (Koricheva et al., 2013). Hedge's d estimates generally follow a normal distribution compared to an odds ratio or correlation

coefficient (Sánchez-Meca et al., 2003), which meets the assumptions of a fixed or random effects model (Koricheva et al., 2013). Additionally, I chose Hedge's d over Hedge's g or Cohen's d because Hedge's d includes a correction factor for small sample size and is minimally affected by sampling variances from paired groups that are unequal. Hedge's d is also suitable when there are as few as five to ten studies (Olkin and Hedges, 1985).

I used a fixed-effects factorial meta-analysis to test the effects of temperature and predator presence on Hedge's d (Brown et al., 2018; Burkepile and Hay, 2006; Lange et al., 2018; Morris et al., 2007; Stockbridge et al., 2020; Worm et al., 2002; Wright, 2019) (Table 2-2). Fixed-effects models assume that all studies share a common effect size (Koricheva et al., 2013). This assumption of a shared common effect size may not be true if the studies involved in the meta-analysis vastly differ (e.g., differ in their methodology or studied species). A random or mixed-effects model could theoretically be used to account for among-study variance; however, these types of models have not yet been developed for a factorial meta-analysis (Gurevitch et al., 2000). I defined cold temperature treatments as the 'control' group as I was primarily interested in the effects of warming on body size. I considered multiple studies, prey species, predator species, or study sites within a paper to be independent (i.e., not sharing the same control group).

Table 2-2 Design of the 2×2 factorial meta-analysis used in this study.

	Predator Absent	Predator Present
Control	Prey size at a cold temperature	Prey size at a cold temperature
Experimental	Prey size at a warm temperature	Prey size at a warm temperature

To calculate the effect sizes of temperature, predator presence, and of the interaction between temperature and predator presence, I followed the methods and equations outlined by

Gurevitch et al. (2000). For a 2×2 factorial meta-analysis, the traditional equation for Hedge's d, and its associated variance, are modified to incorporate all four treatments (Olkin and Hedges, 1985). Using the 'metafor' package (Viechtbauer, 2020) in R (version 3.6.2; R Core Team, 2019) I created fixed effects models using the function 'rma.uni' for, 1) the overall effect of warming, 2) the overall effect of predator presence, and 3) the interaction between temperature and predator presence. Each of these models generates a single mean effect size and 95% confidence interval.

I specified the calculation for weighted Hedge's d and its variance using restricted maximum likelihood estimation (Law et al., 2016). I also created additional models to examine whether Hedge's d varied between visual vs tactile predators, or between consumptive vs. non-consumptive predator effects.

Effect size equations

I used the effect size equations from Gurevitch et al. (2000) to calculate the effect size of warming (d_W) pooled across predator treatments (Overall effect of warming), the effect of predator presence (d_P) pooled across temperature treatments (Overall effect of predator presence), and their interaction (d_I) as follows:

$$2\text{-}1.a) \qquad d_W = \frac{(\bar{Y}_{WA} + \bar{Y}_{WP}) - (\bar{Y}_{CA} + \bar{Y}_{CP})}{2s} J(m)$$

$$2\text{-}1.b) \qquad d_P = \frac{(\bar{Y}_{WP} + \bar{Y}_{CP}) - (\bar{Y}_{WA} + \bar{Y}_{CA})}{2s} J(m)$$

$$2\text{-}1.c) \qquad d_I = \frac{(\bar{Y}_{WA} - \bar{Y}_{CA}) - (\bar{Y}_{WP} - \bar{Y}_{CP})}{s} J(m)$$

Equations 2-1.a) overall effect of warming, b) overall effect of predator presence, and c) interaction between warming and predator presence.

The mean body size of each group is indicated by $\bar{Y}$, while subscript, s, indicates the pooled standard deviation of the two groups. A correction term for small sample bias is represented by $J(m)$ (Olkin and Hedges, 1985). The degrees of freedom for the overall effects and interaction are $m = N_{WP} + N_{CP} + N_{WA} + N_{CA} - 4$, as all four treatment groups were used in these calculations.

The pooled standard deviation, s, is calculated as:

$$s = \sqrt{\frac{(N_{WP} - 1)(s_{WP})^2 + (N_{CP} - 1)(s_{CP})^2 + (N_{WA} - 1)(s_{WA})^2 + (N_{CA} - 1)(s_{CA})^2}{N_{WP} + N_{CP} + N_{WA} + N_{CA} - 4}}$$

Equation 2-2 Pooled standard deviation used in all effect size calculations.

Where N represents the sample size reported in each paper, and s_{ij} is the standard deviation for each mean value for a given treatment (as indicated by the subscripts) for a given study.

A negative value for d_W or d_P means that body size decreased with warming or with predator presence, respectively. I address the interpretation of d_I in a separate section below.

The sampling variances for d_W and d_P are:

$$2\text{-}3.a) \qquad \hat{s}^2(d_W) = \left[\frac{1}{N_{WP}} + \frac{1}{N_{CP}} + \frac{1}{N_{WA}} + \frac{1}{N_{CA}} + \frac{d_W^2}{2(N_{WP} + N_{CP} + N_{WA} + N_{CA})}\right]\frac{1}{4}$$

$$2\text{-}3.b) \qquad \hat{s}^2(d_P) = \left[\frac{1}{N_{WP}} + \frac{1}{N_{CP}} + \frac{1}{N_{WA}} + \frac{1}{N_{CA}} + \frac{d_P^2}{2(N_{WP} + N_{CP} + N_{WA} + N_{CA})}\right]\frac{1}{4}$$

17

The sampling variance for the interaction, d_I is:

$$2\text{-}3.c) \quad \hat{s}^2(d_I) = \frac{1}{N_{WP}} + \frac{1}{N_{CP}} + \frac{1}{N_{WA}} + \frac{1}{N_{CA}} + \frac{d_I^2}{2(N_{WP} + N_{CP} + N_{WA} + N_{CA})}$$

Equations 2-3. Calculations for sample variances for, a) the overall effect of warming, b) the overall effect of predator presence, and c) the interaction between warming and predator presence.

For addressing the effects of predation effects and foraging mode I did not pool across warming treatments. Following the methods and from Gurevitch et al. (2000), dividing the effect sizes for studies based on a variable of interest (in my case, predation effects and foraging mode) means I cannot pool across treatments in the same way. Using a different set of equations provided by Gurevitch et al. (2000), I calculated effect sizes for warming with predator present (wP) for each study. I then grouped these effect sizes based on a predator effect or foraging mode. For each grouping I used a fixed-effects model to assess the effect of predation effects and foraging mode on prey body size. I then compared the mean effect sizes and 95% confidence intervals for each predation effect and foraging mode to see which factor had the greatest influence on prey body size. The treatment groups were denoted as cold (C), warm (W), predator absent (A), predator present (P):

$$d_{wP} = \frac{(\bar{Y}_{WP} - \bar{Y}_{WA})}{s} J(m)$$

Equations 2-4. Effect size calculations for the effect of warming with predator presence.

The degrees of freedom, m, for the effect of warming with predators present $m = N_{WP} + N_{CP} - 2$. A negative value for this effect indicates body size decreased with warming. The pooled standard deviation, s, and $J(m)$ is a correction term for small sample bias are calculated the same as shown in the equations presented above.

I used the statistic Q to evaluate the homogeneity of mean effect sizes (Hedge's d) within and between studies (Koricheva et al., 2013). This statistic is calculated by the 'rma.uni' function in R and is provided in the R output. The test for homogeneity addresses the overall similarity, or lack thereof, in the outcomes of various studies (Koricheva et al., 2013). The null hypothesis for the total heterogeneity of my study is that all studies are a homogenous sample from a population of studies with a true effect size (Koricheva et al., 2013). There is an observed X^2 distribution in a parametric fixed-effects model for the homogeneity statistic Q (Gurevitch and Hedges, 1999; Olkin and Hedges, 1985). The total heterogeneity among studies, Q_T, can be partitioned into between, Q_B, and within, Q_W, study heterogeneity. Testing the heterogeneity between studies provides information on whether groups of studies differ based on their mean effect size (e.g., if prey body size differs in its response to consumptive versus non-consumptive predator effects).

I also calculated the I^2 statistic, which is an alternative measure of heterogeneity. I^2 is the comparison of Q_T to its expected value under the assumption of homogeneity; in other words, the I^2 is the percentage of total heterogeneity that can be assigned to the variance between studies (Koricheva et al., 2013; Nakagawa and Cuthill, 2007). I^2 values <25% suggest high consistency among studies.

Interpretation of the interaction effect size, d_I

The Hedge's d calculation for the interaction between two overall effects (Equations 2-1.c) subtracts the 'effects of warming in the presence of predators' from the 'effects of warming in the absence of predators' (Crain et al., 2008; Gurevitch et al., 2000). If this effect size is equal to zero (Box 2-1, Figure 2-1), this result implies that the presence of predators does not change the main effect of warming (Gurevitch et al., 2000). This result is analogous to a non-

statistically significant interaction term in an ANOVA and implies that the combined effect of warming and predator presence is equal to the sum of the individual components (additive effect). If the 'effect of warming in the absence of predators' is greater than the 'effect of warming in the presence of predators', the interaction effect size will be greater than zero (Equation 2-1.c, Box 2-1, Figure 2-1), and the interpretation in this case is that in the presence of predators the effect of warming is less intense than when predators are absent (antagonistic effect). Finally, if the 'effect of warming in the absence of predators' is less than the 'effect of predators in the presence of predators', the interaction effect size will be less than zero (Equation 2-1.c, Box 2-1, Figure 2-1), meaning that in the presence of predators the effect of warming is more intense than when predators are absent.

(A) Additive | Antagonistic | Synergistic
Predator absent ○
Predator present ●

Warming: $(\bar{Y}_{WA} + \bar{Y}_{WP}) - (\bar{Y}_{CA} + \bar{Y}_{CP})$
$= (3 + 1) - (4 + 2)$
$= -2$

Predators: $(\bar{Y}_{WP} + \bar{Y}_{CP}) - (\bar{Y}_{WA} + \bar{Y}_{CA})$
$= (1 + 2) - (3 + 4)$
$= -4$

Interaction: $(\bar{Y}_{WA} - \bar{Y}_{CA}) - (\bar{Y}_{WP} - \bar{Y}_{CP})$
$= (3 - 4) - (1 - 2)$
$= 0$

(B)
Warming: $(\bar{Y}_{WA} + \bar{Y}_{WP}) - (\bar{Y}_{CA} + \bar{Y}_{CP})$
$= (4 + 2) - (3 + 1)$
$= 2$

Predators: $(\bar{Y}_{WP} + \bar{Y}_{CP}) - (\bar{Y}_{WA} + \bar{Y}_{CA})$
$= (2 + 1) - (4 + 3)$
$= -4$

Interaction: $(\bar{Y}_{WA} - \bar{Y}_{CA}) - (\bar{Y}_{WP} - \bar{Y}_{CP})$
$= (4 - 3) - (2 - 1)$
$= 0$

(C)
Warming: $(\bar{Y}_{WA} + \bar{Y}_{WP}) - (\bar{Y}_{CA} + \bar{Y}_{CP})$
$= (2 + 4) - (1 + 3)$
$= 2$

Predators: $(\bar{Y}_{WP} + \bar{Y}_{CP}) - (\bar{Y}_{WA} + \bar{Y}_{CA})$
$= (4 + 3) - (2 + 1)$
$= 4$

Interaction: $(\bar{Y}_{WA} - \bar{Y}_{CA}) - (\bar{Y}_{WP} - \bar{Y}_{CP})$
$= (2 - 1) - (4 - 3)$
$= 0$

Body size

Cold Warm Cold Warm Cold Warm

(D) Antagonistic / Additive / Synergistic

(E) Antagonistic / Additive / Synergistic

Hedge's d

(F) Synergistic / Additive / Antagonistic

Warming Predators Interaction

Figure 2-1 A conceptual approach based on Figure 1 in Crain et al. (2008) to interpret the data on interaction types for two-by-two factorial studies (A-C) and corresponding effect sizes of Hedge's d (D-F) for individual and interactive effects. The grey boxes provide the numerator components to the overall effect size equations for warming, predator presence, and their interaction. These boxes merely indicate the direction of the corresponding effect sizes (positive, negative, or zero; D-F) for the example of an additive model and use the values from the figures in the additive column (A-C). The direction for antagonistic and synergistic interactions can also be calculated using the values from the figures in the antagonistic and synergistic columns (A-C). Presented on the right are the three effect size scenarios (D-F) that correspond to the three data scenarios (A-C), with positive effect size values for both warming and predator presence (D calculated from A), contrasting effect size values for warming and predators (E calculated from B), and negative effect size values for both warming and predator presence (F calculated from C).

Assessing sample size, temperature treatments, and publication bias

I used linear regressions to test the effect of temperature range on the effect size (Hedge's d). If there is no correlation between temperature range and effect size among studies, this suggests that temperature range explains little variance in the calculated effect sizes.

To assess potential publication bias I created a funnel plot, which assesses standard error against the residual values. If the funnel plot is symmetric it suggests that there is no publication bias, with higher precision studies plotted near the mean and low precision studies spread evenly on both sides of the mean (Duval and Tweedie, 2000). If a funnel plot is asymmetric it suggests publication bias is present and indicates a relationship between treatment effect estimate and study precision (Duval and Tweedie, 2000). I also conducted a linear regression to test the effect of sample size within each paper against the calculated effect size (Rothstein et al., 2005). Here, if there is a relationship between sample size and effect size, whereby a higher sample size is correlated with a larger effect size, it suggests some form of publication bias in that studies with a smaller sample size may not be as robust (e.g., smaller scale studies may lack the necessary funding, and or, equipment to produce accurate results) (Rothstein et al., 2005).

Assessing power

I conducted a power analysis with function 'mpower' in the package 'metapower' in R version 3.6.2 (Griffin, 2020; R Core Team, 2019). This function allows the input of a predicted effect size, mean sample size within papers, number of papers, and type of effect size metric (ex. Odds ratio or standardized mean differences). With the planned use of a standardized mean difference (SMD) for our effect size metric (as indicated by es_type = "d" in the 'mpower' function), I used 0.3 as my predicted effect size (Figure 2-2). The specific number is arbitrary as

it was challenging to find consensus in the literature for a given SMD effect size for the effect of warming and the effect of predation on prey body size, but the 'mpower' function autogenerates a range of effect sizes around the predicted value (0.15-0.6). The mean sample size within papers was approximately based on the average sample size found in papers (assessed using ~10-20 papers) that tested variables independently (i.e., warming or predator presence), or together (i.e., warming and predator presence). The number of papers was chosen based on suggested sample sizes to use for pilot studies (i.e., $n \geq 30$) (Hogg et al., 2013). A random model takes into account both within and between sample variance (i.e., a high heterogeneity assumes a large percentage of the variance is due to the variance between studies) and is thus often favored over a fixed model that only takes into account within sample variance (Koricheva et al., 2013); however, a fixed model is most similar to the meta-analysis I conducted given the two-by-two factorial meta-analysis approach (Gurevitch et al., 2000).
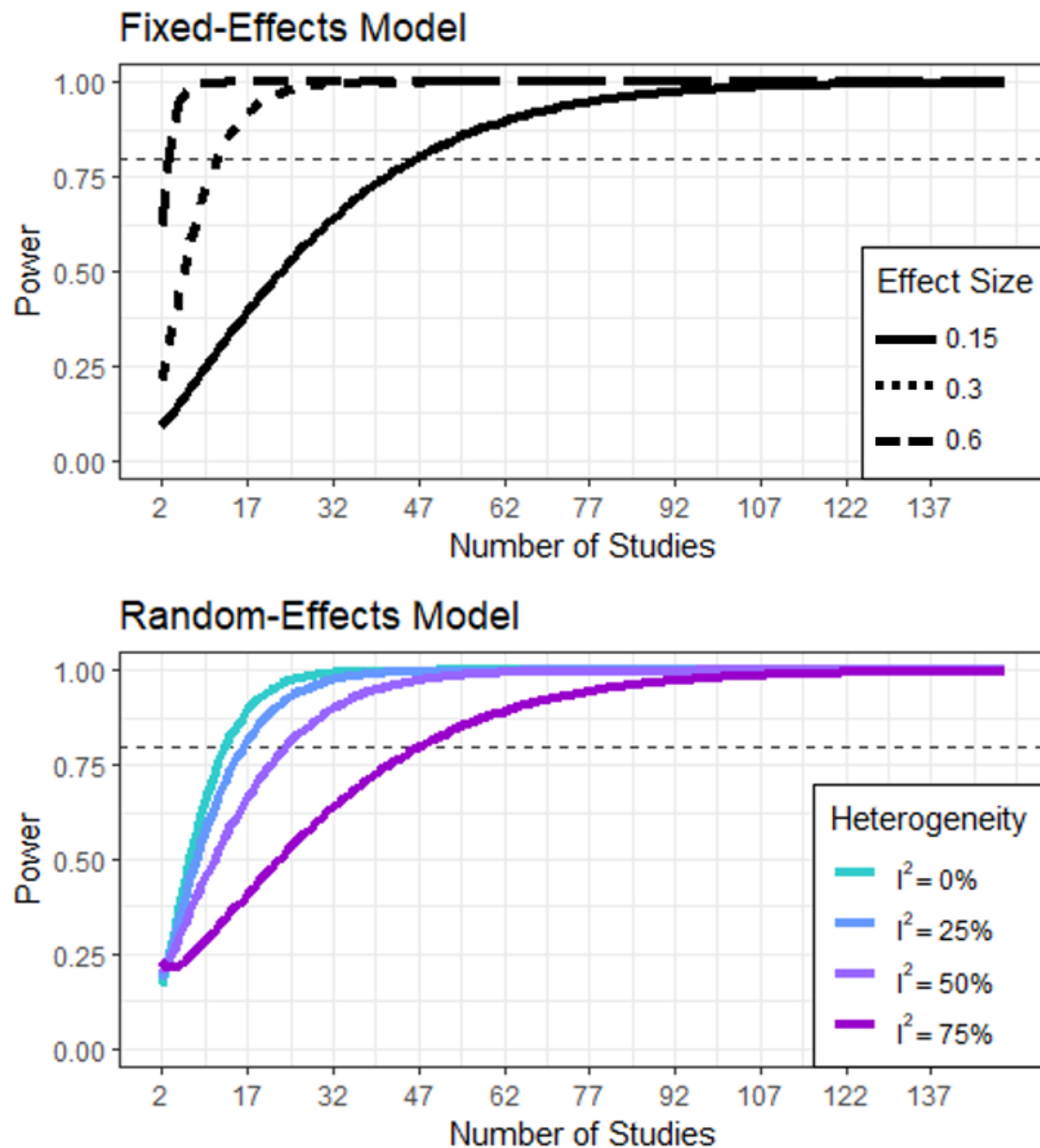
Figure 2-2 The power analysis for effect size estimates for the effects of, 1) warming, 2) predator presence, and 3) warming and predator presence on prey body size.

2.4   Results

2.4.1   The dataset

From the 14 papers used in my analyses (Table 2-3), the data encompassed 12 prey species and 10 predator species, and all prey and predator species were aquatic. Among all studies, the most common prey was zooplankton, and the most common predator was fish. This led to visual predators being the most reported, with the most common type of predator effect being non-consumptive. Non-consumptive predator effect treatments included the use of predator water or extracted kairomones added to a prey apparatus or the placement of a barrier between predator and prey. Most experiments used juvenile or larval stages of predators and adult or juvenile stages of prey. Most studies were conducted in the summer.

Table 2-3 A total of 14 studies were used in the analysis with some having multiple effect sizes calculated based on defined, independent groups.

Article	Number of effect sizes
Cavalheir et al. 2019	4
Costa and Kishida 2015	1
Gilbert 2011	2
Grigaltchik et al 2016	4
Huss et al 2010	2
Maclennan and Vinebrooke 2016	1
Pavon-Meza et al 2007	1
Peckarsky et al 2001	1
Scherer et al 2013	1
Tseng et al 2018	3
Tseng and O'Connor 2015	2
Weetman and Atkinson 2002	1
Weetman and Atkinson 2004	4
Zhao et al 2014	2

2.4.2 Main and interaction effects of warming and predator presence on prey body size

The effect size for warming was not significantly different than zero (d = 0.0151, CI = -0.025 to 0.056, k = 26). The effect size for predator presence was positive and significantly different than zero (d = 0.295, CI = 0.254 to 0.335, k = 26; Figure 2-3). The effect size for warming was significantly different from the effect size for predator presence, as indicated by non-overlapping 95% confidence intervals. The interaction effect size did not differ significantly from zero (d = -0.043, CI = -0.123 to 0.037, k = 26), indicating that the combined effects of warming and predator presence is additive, rather than synergistic or antagonistic. The test for heterogeneity was significant for all models suggesting that most of the total heterogeneity can be attributed to between study variance (Table 2-4).
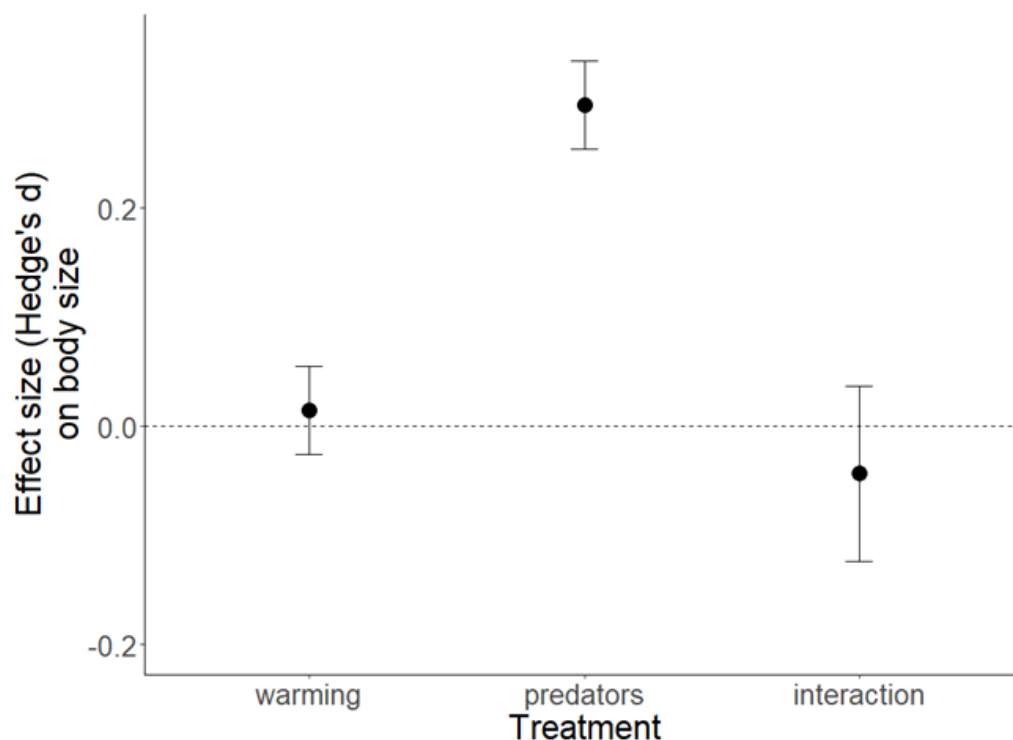
Figure 2-3 The effect sizes and 95 % confidence intervals for the overall effects of warming, predator presence, and their interaction.

Table 2-4 Results from homogeneity tests for the effects of temperature for data shown in Figure 2-3.

Value tested	Treatment	K	Q_T^{**}	I^2 (%)
Body size	Warming	26	791.06	96.72
	Predator presence	26	665.89	96.25
	Interaction	26	66.67	62.50

** $p < 0.0001$

2.4.3 Effects of predation effect and foraging mode on prey body size

The effect size for tactile predators was larger in magnitude compared to all other predator groupings (Figure 2-4). The effect size for tactile predation was negative (d = -1.772, CI = -2.082 to -1.462, k = 3), whereas the effect size for visual predation was positive (d = 0.500, CI = 0.272 to 0.727, k = 13). The effect size for non-consumptive predator effects did not differ significantly from zero; however, that for consumptive predator effects was greater than zero (d = 0.089, CI = 0.029 to 0.150, k = 8). The test for heterogeneity was significant for all effects except for the effect of tactile predation (Table 2-5).
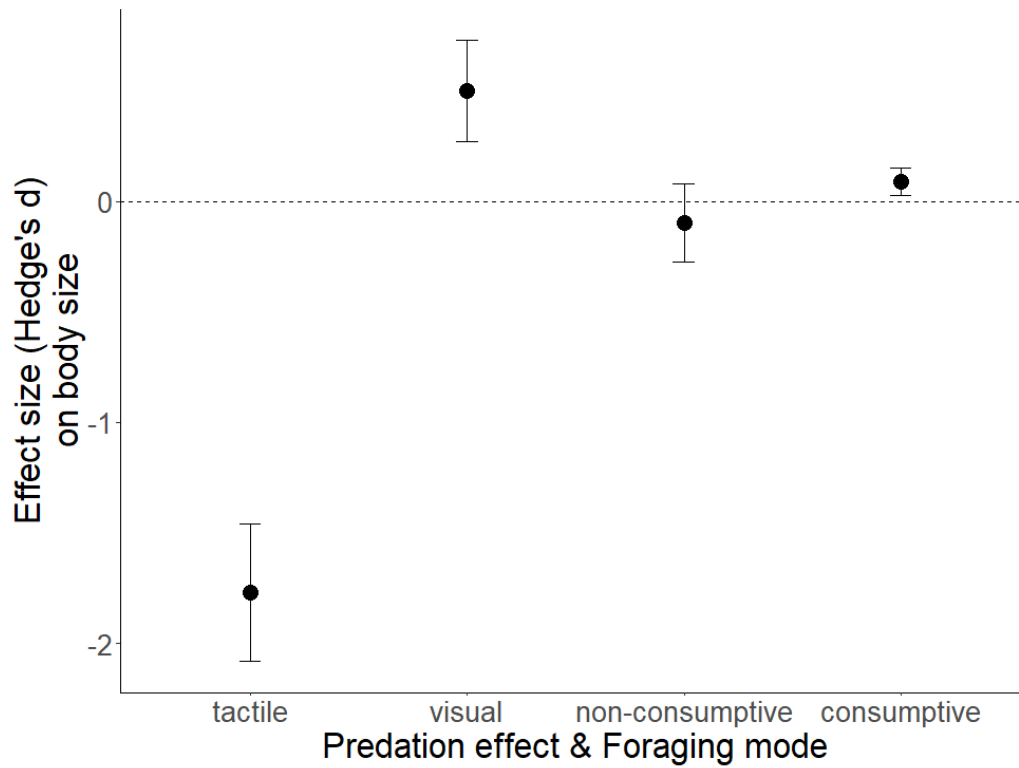
Figure 2-4 The effect sizes and 95% confidence intervals for the main effects of predation effect and foraging mode on prey body size. Indirect and direct predation refer to non-consumptive and consumptive predator effects, respectively.

Table 2-5 Results from homogeneity tests for the effects of temperature for data shown in Figure 2-4.

Grouping tested	Predator	K	Q_T	p	I^2 (%)
Tactile	Present	3	22.81	<0.0001**	91.23
Visual	Present	13	139.10	<0.0001**	91.37
Indirect	Present	18	264.43	<0.0001**	93.57
Direct	Present	8	62.94	<0.0001**	88.88

**p < 0.0001

2.4.4 Assessing publication bias

There is a slight asymetric spread within the funnel plots created from the models on the overall effects of warming and predator presence. There is a larger majority of data points within the right side of the plot for the overall effects of warming (Figure 2-5A) and larger majority of data points falling within the left side of the plot for the overall effects of predator presence (Figure 2-5B). This asymetry suggests some publication bias might be present for the papers used in my study.
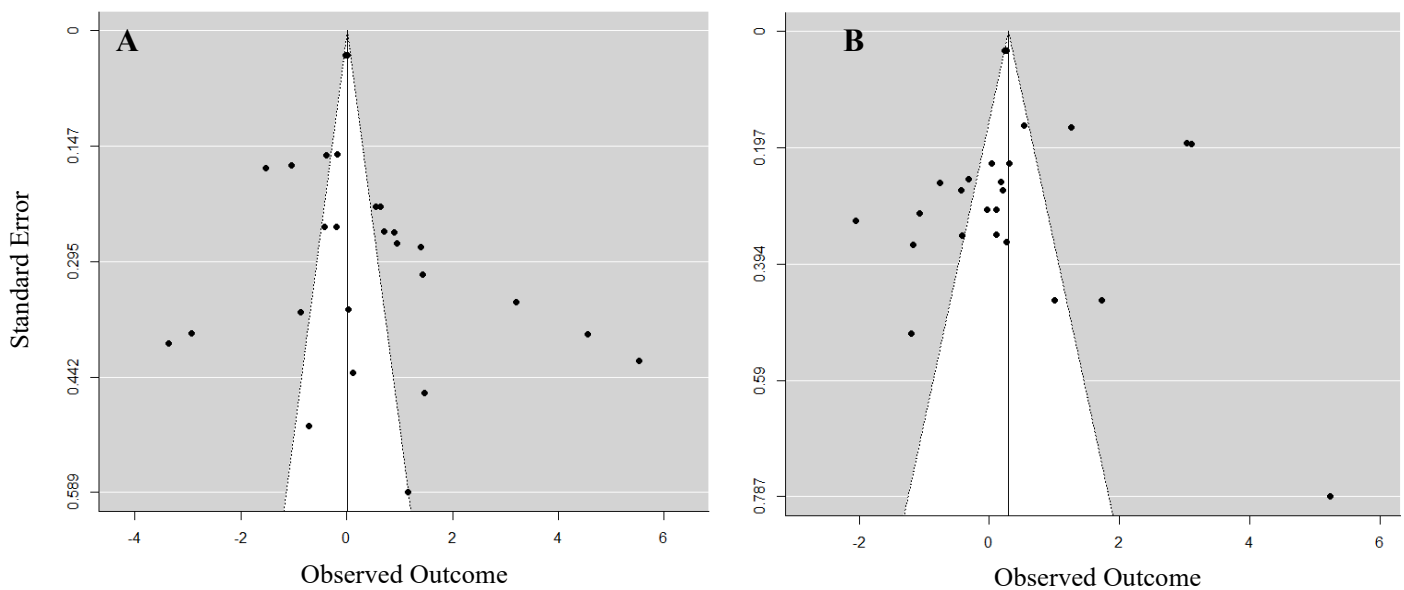
Figure 2-5 Funnel plots of the models for (A) overall effect of warming and (B) overall effect of predator presence displaying relatively asymmetric data points around the mean value of 0, indicating potential publication bias.

Effect of pooled sample size on effect size

There was no significant effect of pooled sample size on the weighted effect size metric (Hedge's d) in the data used in the overall effects model of warming ($F_{(1,24)} = 0.340$, p = 0.565; Figure 2-6A) or in the data used in the overall effects model of predator presence ($F_{(1,24)} = 0.242$, p = 0.627; Figure 2-6B).
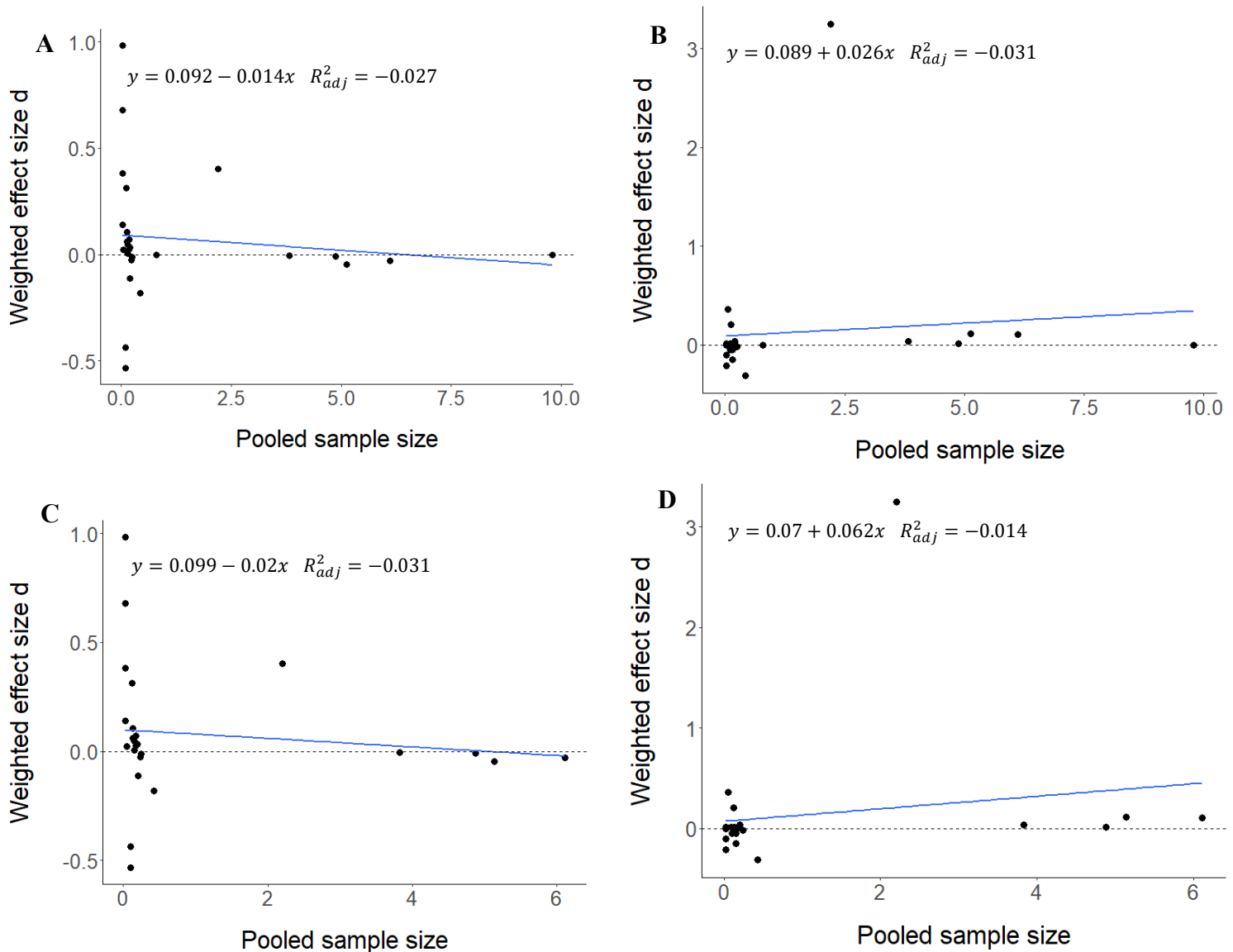
Figure 2-6 Linear regressions for testing the effect of pooled sample size on the weighted effect size metric (Hedge's d). (A) and (C) plot the data of the overall effect of warming with and without a study with a sample size of 1000, respectively. (B) and (D) plot the data of the overall effect of predator presence with and without a study with a sample size of 1000, respectively.

However, there was one study that had a sample size of 1000 that could bias this linear regression. With the removal of this study, there is still no significant effect of pooled sample size on the weighted effect size metric for either the data used in the overall effects model of warming ($F_{(1,22)} = 0.312$, $p = 0.582$; Figure 2-6C) or for the data used in the overall effects model of predator presence ($F_{(1,22)} = 0.689$, $p = 0.420$; Figure 2-6D).

2.4.5   Effect of temperature range on calculated effect sizes

There was no effect of temperature range on the calculated overall weighted effect sizes from warming ($F_{(1,22)} = 0.931$, $p = 0.345$; Figure 2-7A) or predator presence ($F_{(1,22)} = 1.095$, $p = 0.307$; Figure 2-7B).
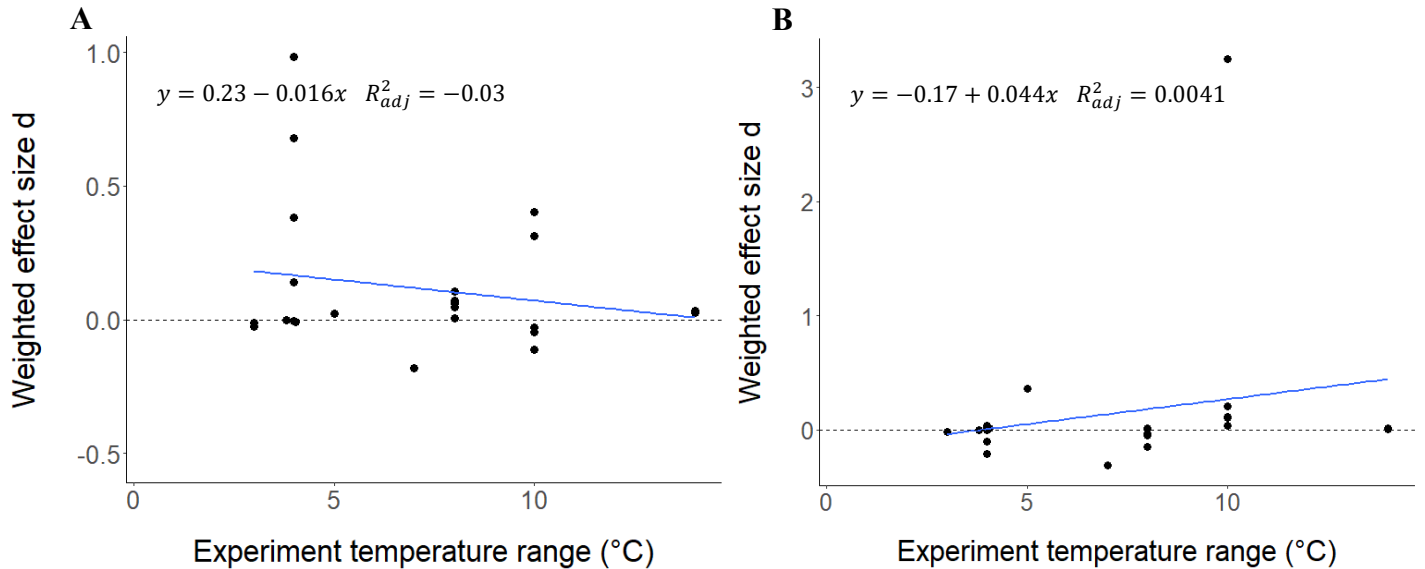
Figure 2-7 Linear regressions for testing the effect of experiment temperature range (°C) between studies on the weighted effect size metric (Hedge's d). (A) the data of the overall effect of warming, and (B) the data for the overall effect of predator presence.

2.4.6   Assessing power

For effect sizes close to or below 0.15, my power analysis suggests I would need approximately 47 papers to achieve 85% power (Figure 2-2). For effect sizes close to 0.3, my power analysis suggests I would need approximately 15 papers to achieve 85% power. My meta-analysis consisted of 14 papers, with effect sizes ranging approximately between -0.04 to 0.3 for the overall effects off warming, predator presence and their interaction and -1.8 to 0.5 for the effects of predator characteristics. Based on the power analysis my study may not have enough

power to detect the main effects of warming and predator presence on prey body size (discussed below).

2.5   Discussion

Overall, the meta-analysis demonstrated that warming did not change prey body size and predator presence increased prey body size. The interaction effect size between warming and predator presence was zero. This result suggests that the combined effect of warming and predator presence caused an overall increase in prey body size (neutral effect of warming plus a positive effect of predator presence). Surprisingly, prey body size decreased in the presence of tactile predation and increased in the presence of visual predation. Also, in contrast to predictions, non-consumptive predator effects decreased prey body size while consumptive predator effects increased prey body size.

Warming

I predicted that warming would significantly decrease prey body size. For example, Sotoyama et al. (2018) studied the effects of temperature on body size and body composition of juvenile yellowtail fish and found warming to cause juvenile fish to grow significantly less and have significantly lower body protein content and lipid content. Using organism body size as a proxy for organism quality, my results suggest that warming does not impact organism quality. A possible explanation for why the overall effect of warming in my study was relatively neutral could be a result of the low number of papers used in my meta-analysis producing insufficient power to detect an effect. This can lead to Type II error, which is the non-rejection of a false null hypothesis (Underwood and Chapman, 2003). The absolute value for the overall effect size of

warming on body size was 0.015, which is much lower than the effect size of 0.15 that requires

approximately 47 papers to achieve 85% power (as based on my power analysis). Therefore, the

detection of a neutral effect of warming on prey body size may be false and a larger sample size

of papers is needed to accurately test the effects of warming. A neutral effect of warming could

also be caused by a specific response from zooplankton compared to other organisms (Atkinson

and Sibly, 1997; Blanckenhorn, 2000), with the prey species in my study being primarily

zooplankton.

Predator presence

Prey body size increased in the presence of predators. This unexpected result may be due

to the number of visual vs. tactile predators included in the meta-analysis. There was a

significant positive effect of visual predation on prey body size and a higher prevalence of visual

predators in my dataset than tactile predators. The large majority of juvenile or larval predators

in my data could help explain why prey body size under visual predation went against

predictions. Juvenile or larval stages may be gape-limited and only capable of selecting smaller

prey individuals as opposed to larger prey (Hart and Bychek, 2011). The opposite trend observed

for tactile predation may be a result of low sample size. Three data points is likely not enough to

accurately detect an effect of tactile predation.

The overall positive effect of (mostly visual) predator presence on prey body size could

be due to size-selective predation. For example, Sell (2000) reported that tactile aquatic predators

prefer to eat small prey individuals, while Viitasalo et al. (2001) found that visual aquatic

predators to remove large prey individuals. This means size-selection predation under tactile

predators resulted in a population of larger prey individuals compared to visual predators causing

a population of smaller prey individuals. However, the additional findings from Sell (2000) provide evidence for the multi-generational effects of aquatic predators. Aquatic predators can decrease prey body size because of the non-consumptive effects of predators selecting prey with traits of increased growth rates and decreased size at maturity (Sell, 2000). These observed variations of the effects of predator presence on prey body size will alter prey quality and impact the combined effects of predator presence and warming.

Predator presence & warming

There are few studies that have investigated the combined effects of warming and predator presence on prey body size. Some examples of studies (used in my meta-analysis) involve the effects of warming and presence of salamander larvae predators on tadpole body width (Costa and Kishida, 2015), and the effects of warming and presence of crab predators on scallop body mass (Guerra et al., 2012). Using a lab experiment, Costa and Kishida (2015) found warming to increase predation rates on tadpoles, but instead of observing a predicted decrease in tadpole body size they observed an increase in body size. Also using a lab experiment, Guerra et al. (2012) found that warmer water temperatures increased scallop growth and body mass and predator presence caused prey to reallocate energy to muscle growth (i.e., body mass). These two studies suggest that aquatic prey may increase body size when subject to both predator presence and warming. Gape-limited predators can remove smaller individuals from the population, leaving behind larger individuals to repopulate (Paine, 1976), and warming can cause increased growth rates of these larger individuals (Atkinson and Sibly, 1997). In the case of Guerra et al. (2012), the observed increased growth rates in scallops exposed to warming would suggest decreased development time resulting in smaller size at maturity (Atkinson and Sibly, 1997).

However, the observed increase in prey size in this analysis, potentially caused by consumptive predation, may provide evidence for predator presence having a stronger effect than that of warming on prey body size. Additionally, warming may exacerbate the effects of predator presence, if larger prey left to repopulate reach maturity sooner and begin reproducing sooner because of increased growth rates.

My study is one of the first to apply a meta-analytical approach with the aims of understanding potential trends in the literature for the individual and interactive effects of warming and predator presence on prey body size. My tentative results indicate an additive effect of warming and predator presence on prey body size. Specifically, for prey in the presence of both warming and predators, predator presence will have a stronger effect than warming on prey body size and will increase prey body size as a result. The finding of increased body size caused by aquatic predators aligns with the results found in the currently small body of literature on the combined effects of warming and predator presence, but my finding of warming having a neutral effect on body size goes against what is currently known. If the small warming effect size seen here is accurate, a larger number of papers (and thus higher power) is needed to accurately assess if warming influences body size. The low sample size in my study makes the results of my meta-analysis slightly inconclusive. Further data collection is needed to better understand the effects of warming and predator presence on prey body size. Improving our understanding of the combined effects compared to the effects of warming and temperature separately is more biologically meaningful and will help inform how organism quality and consumer quality will change with ongoing climate warming.

Chapter 3: Testing the effects of temperature on nutrient quality, quantity, and cascades in food webs

This chapter is another version (with some variation in methodology and hypotheses) of a paper that involved collaborations with Madeline Fung, Ian Forster, and Michelle Tseng. My role was to help count zooplankton populations, measure insect larvae growth rates, and feed and maintain insect larvae populations. I was not involved in the growing or quantifying of algae.

3.1   Introduction

3.1.1   The effects of temperature on individuals

Understanding how individuals respond to environmental change can help us understand the functioning and interactions of ecological communities (Begon et al., 1990; Odum and Odum, 1997) and help predict the effects of climate change on communities. Examples of individual-level responses to the environment include well-studied aspects like changes in organism growth rate (Arft et al., 1999; Cantin et al., 2010; Jump et al., 2006; Koricheva et al., 1998), fecundity (Arendt, 2011; Kjesbu et al., 1998; Rall et al., 2012; Saavedra et al., 2003) or behaviour (Huey et al., 2012, 2009; Nagelkerken and Munday, 2016; Zvereva and Kozlov, 2006), but can also include changes in organism nutrient composition (Arcus and Mulholland, 2020; Dijkstra et al., 2012; Fuschino et al., 2011; Hixson and Arts, 2016; O'Donnell et al., 2019). Nutritional composition shifts have the potential to significantly affect the fitness (i.e., growth and fecundity) of species that consume these organisms (Harvey and Moore, 2017; Savage et al., 2004), but have received far less attention than other organism responses (Rosenblatt and Schmitz, 2016).

We currently know that environmental change can alter the nutrient composition of resources or prey species through changes in key nutrients and biomass. For example, warmer temperatures can decrease grass nutrient quality (increased C:N ratios), leading to decreases in the body size of grasshopper consumers (Laws and Joern, 2013). These body size reductions decrease predation of grasshoppers by wolf spiders (Laws and Joern, 2013). This example highlights the general understanding of how environmental change, such as a change in temperature, can affect the functioning of food webs via shifts in prey or resource quality.

The effects of temperature on resource quality are particularly evident in aquatic systems. Phytoplankton are the largest photosynthesizing biomass on earth (Falkowski et al., 1998) and they are a key direct and indirect resource for higher aquatic trophic levels (Field et al., 1998). In some phytoplankton species, temperature increases can decrease quantities of beneficial polyunsaturated omega-3 fatty acids (Breuer et al., 2013; Fuschino et al., 2011; Guschina and Harwood, 2009; Hixson and Arts, 2016; Sikora et al., 2014). These fatty acids are essential for proper growth and development in phytoplankton as well as in most aquatic organisms (Arts et al., 2001; Kaur et al., 2014; Spector and Kim, 2015). The predicted decrease in phytoplankton polyunsaturated omega-3 fatty acid content with global warming (Fuschino et al., 2011; Hixson and Arts, 2016; O'Donnell et al., 2019) could have serious implications for the health of higher trophic levels. For example, Hixson and Arts (2016) used a modelling approach to predict that with a 2.5 °C increase in water temperature, we expect a global reduction in polyunsaturated omega-3 fatty acid productions, with eicosapentaenoic acid (EPA) reduced by 8.2% and docosahexaenoic acid (DHA) reduced by 27.8% (Hixson and Arts, 2016). Despite the known effects of temperature on phytoplankton fatty acid productions, whether temperature-mediated

changes in these key nutrients affects the productivity of higher trophic levels in aquatic systems has rarely been tested empirically.

3.1.2   Study objectives

That empirical evidence in terrestrial and aquatic systems for shifts in organism quality caused by warming provides rational for an exploratory experiment testing the hypotheses that, (1) warming affects resource quality in a simple aquatic community (phytoplankton, zooplankton, insect larvae), and that (2) resource quality will have cascading effects and impact consumer fitness. We used fatty acid quantity and composition as a measure of organism quality due to their importance for proper growth and development (Field et al., 1998). To examine the cascading effects of temperature on resource quality, we grew algae (*Scenedesmus obliquus*), zooplankton (*Daphnia pulex*), and insect larvae (*Chaoborus americanus*) at different temperatures and fed each trophic level to the subsequent tier (i.e., *S. obliquus* to *D. pulex* and *D. pulex* to *C. americanus*). We measured fatty acid profiles of *S. obliquus* and *C. americanus*, and productivity in all three species. Algal productivity was quantified by undergraduate researcher Madeline Fung and is not included here. Because of insufficient sample sizes, we were unable to measure *D. pulex* fatty acid quantity and composition. We discuss the effects of a warming climate on nutrient quality in aquatic food webs and the implications that warming and nutrient quality can have on future food web dynamics within and across ecosystems.

3.1.3   Fatty acids in aquatic ecosystems

There are several different classes of fatty acids, and those classified as essential 'long chain polyunsaturated fatty acids' (LC-PUFA) have known health benefits for many animals including

humans (Arts et al., 2001; Kaur et al., 2014; Spector and Kim, 2015). Two omega-3 LC-PUFA

that have important functions are eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic

acid (DHA; 22:6n-3). Deficiencies in EPA and DHA can lead to severe developmental and

neurological disorders (Innis, 2005; Simopoulos, 1999) and general malnutrition (Arts and

Kohler, 2009; Simopoulos, 1999). An omega-6 LC-PUFA of high importance is arachidonic acid

(ARA; 20:4n-6) because of its role in cell structure and its effect on overall organism growth,

development, and cell repair (Tallima and El Ridi, 2018). To produce EPA and DHA, the

structural precursor of alpha-linolenic acid (ALA; 18:3n-3) is altered (Cook and McMaster,

2002). ALA is considered an essential fatty acid as many vertebrates lack the enzymes necessary

to form ALA *de novo* and must consume ALA from an outside source (Cook and McMaster,

2002). Dietary sources of EPA and DHA help organisms to obtain sufficient quantities of these

fatty acids (Parrish, 2009). ARA can be synthesized from the precursor linoleic acid (LIN; 18:2n-

6c). LIN is also considered an essential fatty acid because vertebrates cannot synthesize LIN and

must consume LIN from a resource (Tallima and El Ridi, 2018). Both omega-3 and omega-6

LC-PUFA play an important role in the proper development and growth of organisms, but the

ratio of omega-3:omega-6 LC-PUFA also appears to be important. Lower omega-3:omega-6

ratios contribute to decreased cardiovascular health, cognitive ability, and increased

inflammation (De Meester, 2013; Noori et al., 2011; Nunes et al., 2017; Strandvik, 2011).

Investigating how essential LC-PUFAs change with environmental perturbations is important to

understand the health of organisms under a changing climate. This is especially true because

aquatic ecosystems play a key role in supplying both aquatic and terrestrial systems with LC-

PUFAs.

Algae are the primary source of essential LC-PUFA in both terrestrial and aquatic ecosystems (Field et al., 1998; Gladyshev et al., 2017; Guschina and Harwood, 2009; Hixson et al., 2015; Parrish, 2009) and both freshwater and marine algae produce large quantities of essential LC-PUFA (Field et al., 1998). Primary aquatic consumers such as zooplankton and other invertebrates obtain the bulk of their essential LC-PUFA from algae, but they are also able to convert some shorter-chain fatty acids into essential LC-PUFA in situ (Hixson et al., 2015; Twining et al., 2016). Zooplankton fatty acid profiles can sometimes mimic those of their phytoplankton prey (Brett et al., 2006) but the effect of environmental temperature on essential LC-PUFA production or accumulation in primary consumers has not been well documented.

3.2 Predictions

3.2.1 Effect of temperature on *S. obliquus*, *D. pulex*, and *C. americanus* fatty acids

Temperature and S. obliquus fatty acids

Previous studies have shown that warmer temperatures reduce omega-3 LC-PUFA (e.g., EPA, DHA, ALA) production, and increases saturated fatty acid production (e.g., the saturated fatty acids palmitic acid 16:0 and stearic acid 18:0) (Breuer et al., 2013; Sikora et al., 2014; Vigeolas et al., 2012) in *S. obliquus.* Additional studies support these findings, with warmer temperatures causing a shift from unsaturated fatty acids (UFA), including monounsaturated (MUFAs) and polyunsaturated (PUFAs) fatty acids, to saturated fatty acids (SFA), and a shift from omega-3 PUFA to omega-6 PUFA (Hixson and Arts, 2016; O'Donnell et al., 2019). We therefore expected the same responses here (Box 3-1). For a full list of fatty acid abbreviations, please refer to List of Abbreviations.

Temperature and D. pulex fatty acids

Some studies have shown that *D. pulex* produced more essential LC-PUFA when reared at colder temperatures, but some PUFAs (e.g., ARA) do not change with temperature (Goulden and Place, 1990; Navarro et al., 2019; Schlechtriem et al., 2006; Sperfeld and Wacker, 2012). Based on these results, we expect warming to decrease quantities of omega-3 PUFA (e.g., EPA and ALA) and increase omega-6 LC-PUFA (e.g., LIN) but have no effect on ARA and DHA (Box 1). However, in *D. magna*, the relationship between temperature and essential LC-PUFA production depended on both algal rearing temperature and zooplankton growing temperature (von Elert and Fink, 2018); thus *D. pulex* fatty acid production may depend on algal food type.

Temperature and C. americanus fatty acids

Although there are no studies that specifically assess the temperature effects on *Chaoborus americanus*, increased temperature reduced the production of UFAs and increased the production of SFAs in both terrestrial and aquatic insects (Hixson et al., 2015; Roy et al., 1991; van Dooremalen et al., 2011; Yi et al., 2015). With high accumulation of fatty acids from their diet and minimal synthesis in situ (Hixson et al., 2015; Torres-Ruiz et al., 2007), we expect *C. americanus* to exhibit a similar fatty acid composition to that of the *D. pulex* they are consuming, but at slightly higher quantities (Box 3-1). We expect slightly higher quantities because of nutrient biomagnification at higher trophic levels (Fraser et al., 1989; Goulden and Place, 1993; Jezyk and Penicnak, 1966; Naiman et al., 2002).

Box 3-1 Potential temperature effects on the fatty acid composition of *S. obliquus*, *D. pulex*, and *C. americanus*. We use the relative quantities of unsaturated vs. saturated fatty acids as a measure of organism quality. Long-chain unsaturated fatty acids are typically considered to be more healthful than saturated fatty acids.

Hypothesis 1:
Warming temperatures will affect nutrient quality.

Prediction:
Warming will decrease healthy unsaturated fatty acids (e.g. LC-PUFA) and increase unhealthy saturated fatty acids.
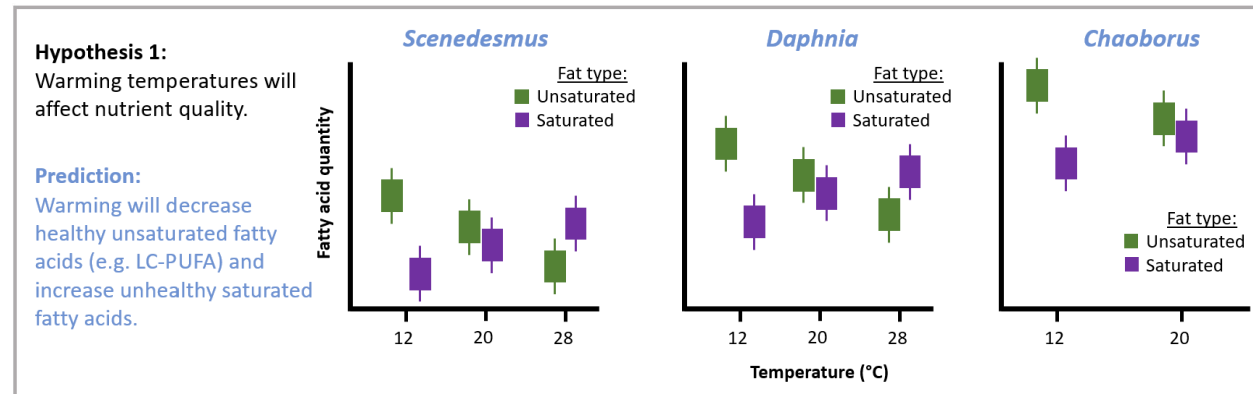
3.2.2   Effect of resource quality and rearing temperature on consumer productivity

Temperature & D. pulex population size

Previous experiments using the same organisms and a similar experimental set-up have generally found that *D. pulex* population size decreases with increasing temperature (Tseng & O'Connor 2015, Tseng et al 2019). We therefore expected *D. pulex* population size to be highest in the 12 ˚C treatment and lowest in the 28 ˚C treatment.

Potential combined effects of D. pulex rearing temperature and algal resource quality on D. pulex population size

Previous studies have shown that with warming, metabolic demands by consumers increase to maintain fitness (i.e., growth and reproduction; Dijkstra et al., 2012; Lemoine and Shantz, 2016) and result in an increased need for high-quality diet (Boersma et al., 2016; Lemoine et al., 2013), even with the associated decrease in body size with warming in some ectotherms (Garzke et al., 2016). Thus, we predict that *D. pulex* fed cold-reared (high-quality) algae will maintain higher population sizes than *D. pulex* fed warm-reared algae. We expect this pattern to hold across *D. pulex* rearing temperature (Box 3-2a).

Potential effect of temperature on C. americanus growth rate

Warmer temperatures accelerate the growth rate of early-instar *C. americanus,* but not late-instar larvae (Frouz et al., 2002; Hanazato and Yasuno, 1989). We expected that the 20°C treatment early-instar *C. americanus* will have higher growth rates compared to the 12°C treatment. *C. americanus* were not reared at 28 ˚C because *C. americanus* experience very rapid

development time at this temperature (Büns and Ratte, 1991) and have low survival rates (Dr. Michelle Tseng, personal observation).

Potential combined effects of C. americanus rearing temperature and D. pulex prey quality on C. americanus growth rate

Aquatic insects can assimilate fatty acids from their diet. For example, some insects obtained high quantities of omega-3 LC-PUFA from *D. pulex* that fed on LC-PUFA rich phytoplankton (Hixson et al., 2015; Torres-Ruiz et al., 2007). We expected *C. americanus* individuals fed a high-quality diet (i.e., *D. pulex* reared at 12 °C and themselves fed *S. obliquus* reared at 12°C) to grow more than *C. americanus* maintained on a poor-quality diet. Therefore, we also expected *C. americanus* grown at 20°C and fed a cold reared diet to have higher growth rate compared to *C. americanus* grown at 12°C and fed a poor-quality diet (Box 3-2b).

Box 3-2 Potential combined effects of diet quality and rearing temperature on the *Daphnia* population size and *Chaoborus* growth rate.

Hypothesis 2:
Diet type will have cascading effects and impact fitness.

Daphnia

Diet type:
- Cold reared
- Ambient reared
- Warm reared

Population size

Temperature (°C)
12   20   28

Prediction 2a:
Warming and warmed reared (nutrient poor) diets will decrease *Daphnia* population size.

Chaoborus

Diet type:
- Cold reared
- Ambient reared

Growth rate

Temperature (°C)
12   20

Prediction 2b:
Warming will increase *Chaoborus* growth rates while warmed reared (nutrient poor) diets will decrease growth rates.

3.3   Methods

3.3.1   Study organisms

A common genus of freshwater algae, *S. obliquus* are found around the world in freshwater bodies (Trainor, 1998). The reproductive characteristics of *S. obliquus* include asexual reproduction through formation of autospores and *S. obliquus* can grow as single cells or colonies (Lürling, 2009). A frequent consumer of *S. obliquus* is the zooplankton, *Daphnia pulex,* with a diet for *D. pulex* comprising primarily of algae (Ebert, 2005). *D. pulex* are filter feeders found globally in lakes and ponds and can experience a large range of temperatures (Ebert, 2005; Mackas, 1992). The reproductive characteristics of *D. pulex* include parthenogenic or sexual reproduction with a generation time (i.e., time needed to develop into a state capable of producing offspring) of approximately 17-20 days (Chen and Stillman, 2012; Ebert, 2005). In turn, *D. pulex* is often predated upon by the midge, *Chaoborus americanus,* but *C. americanus* diet varies with development (Fedorenko, 1975). The first and second-larval instars of *C. americanus* mainly consume small zooplankton while older larval stages primarily consume larger copepods and cladocerans (Fedorenko, 1975). The consumption behaviour of *C. americanus* also changes with development, with *C. americanus* decreasing feeding prior to ovipositing (Moore, 1986). *C. americanus* development takes approximately one year from egg to adult (Fedorenko and Swift, 1972) and adults only live a few days before dying (Cressa and Lewis, 1986).

3.3.2   Source of study organisms

We conducted a laboratory experiment to test the effects of temperature-mediated changes in diet quality on the fitness of zooplankton (*Dapnia pulex*) and insect larvae

(*Chaoborus americanus*). Thousands of *D. pulex* and hundreds of third-instar *C. americanus* were collected using hand nets from the Experimental Ponds Facility at the University of British Columbia on May 14th, 2019. *C. americanus* developmental stage was estimated as third-instar based on published studies of *C. americanus* instar lengths (Carter and Kwik, 1977; Fedorenko and Swift, 1972). *D. pulex* and *C. americanus* were brought back to the lab and kept separately in 10 L white plastic bins filled with pond water and held at ambient temperature (20 °C). We obtained *Scenedesmus obliquus* (CPCC 5) from the Canadian Phycological Culture Centre in 2019. Laboratory cultures of *S. obliquus* were maintained in COMBO medium (Kilham et al., 1998) in 1L glass bottles at 12 ˚C.

3.3.3   *Scenedesmus obliquus* treatments

Scenedesmus obliquus were reared at 12 ˚C, 20 ˚C, and 28 ˚C in light and temperature-controlled incubators (Panasonic MIR-154, 12 hr light; 24W LED, SunBlaster Horticultural Lighting; ~400 µmol m^{-2} s^{-1}), with two incubators per temperature treatment. We used eight replicate algae bottles per treatment (total n = 48). We aerated the algae bottles using filtered air (0.3 µm in-line HEPA-VENT filter). For a full illustration of the apparatus and number of replicates per treatment, please see Figure 3-1.

3.3.4   *Daphnia pulex* treatments

We selected *D. pulex* individuals haphazardly from the culture and placed 30 into each of 180 glass jars (750 mL) each containing 650 ml of COMBO. Thirty jars were randomly assigned to one of the six temperature-controlled incubators (~60 jars per temperature treatment). *D. pulex* jars within each temperature treatment were assigned one of three algal food types: algae reared

at 12 ˚C, 20 ˚C, or 28 ˚C (~20 replicate jars per algal food type, but see *C. americanus feeding treatments* below for exceptions).

Daphnia pulex feeding schedule

During the experiment, *D. pulex* were fed three times per week. At each feeding, approximately 3/5 of the contents of each of the eight algal populations per temperature treatment were poured out into a single plastic bucket and *D. pulex* were fed set concentrations of this pooled algae. Algal rearing bottles were refilled with COMBO. Previous studies have shown that for this type of *D. pulex* rearing environment (starting population size of 30, 750mL jar size), an algal feeding density of 1×10^6 cells per mL was appropriate to minimize resource limitation (Tseng et al., 2018). Algae density was quantified using an imaging flow cytometer (FlowCAM 8400, Fluid Imaging Technologies, Maine USA). Specifications for the FlowCam runs were: 0.4 μL samples, 20x objective, FOV50 flow cell, flow rate = 0.3mL/min. *D. pulex* jars were haphazardly repositioned within each incubator after each feeding. Fifty percent of the COMBO media in the *D. pulex* jars was replaced every two weeks.

Daphnia pulex data collection

Daphnia pulex populations were manually counted at the end of each month to test the effects of resource quality on consumer fitness. All contents of each replicate jar were poured onto a white tray and individual *D. pulex* were counted and transferred back into the same jar using a 3mL pipette. One-third of the COMBO media from each jar was replaced with fresh COMBO at each census.

3.3.5 *Chaoborus americanus* treatments (long-term experiment)

Immediately after collection from the ponds, *C. americanus* were placed individually into 250ml beakers filled with a 50:50 ratio of COMBO (Kilham et al., 1998) and filtered pond water. These beakers were held at 12°C until the start of the experiment. After a two-week holding period, *C. americanus* were randomly assigned to either a 12 ˚C or the 20 ˚C temperature treatment and acclimated to these temperatures for 1 week (30 replicates per treatment). Any *C. americanus* larvae that died in the first week of the experiment was replaced.

Chaoborus americanus feeding schedule

Chaoborus americanus were fed three times per week. *C. americanus* were either fed *D. pulex* reared at 12 ˚C and fed algae reared at 12 ˚C (hereafter D12A12), or fed *D. pulex* that were reared at 20 ˚C and fed algae reared at 20 ˚C (hereafter D20A20). The goal of these *D. pulex-C. americanus* feeding combinations was to examine whether the effects of *D. pulex* diet type on *C. americanus* growth rates was affected by *C. americanus* rearing temperature. Of the 60 *D. pulex* jars per temperature treatment, twelve of these were used for *C. americanus* feeding. This arrangement allowed *D. pulex* reared under the same conditions as experimental *D. pulex* to be fed to *C. americanus* without reducing the size of the experimental *D. pulex* jar populations. Although we did not plan to feed *D. pulex* reared at 28 ˚C to *C. americanus*, we still set aside twelve jars from the 60 jars of *D.pulex* reared at 28 ˚C to have equal number of replicates for all *D. pulex* temperature and diet treatments. There was also the rare time that there were too few *D. pulex* from 12 ˚C and 20 ˚C to complete *C. americanus* feedings on a given day and feedings were supplemented using the 28 ˚C feeder jars.

At each feeding, we assessed *C. americanus* health (i.e., alive, dead, alive-lethargic, or alive-pupated) and if they had eaten the *D. pulex* from the previous feeding session. If individual *C. americanus* had died or pupated, they were removed from the experiment. A single *D. pulex* of equal or smaller size than the *C. americanus* gape width (approximated) was fed to each *C. americanus* (Kruppert et al., 2019). If the *C. americanus* did not feed from the previous feeding, the *D. pulex* was removed and a new *D. pulex* was provided. The removal and replacement of uneaten *D. pulex* was purposeful to avoid characteristics (e.g., size or behaviour) of an individual *D. pulex* biasing consumption rates by *C. americanus* (Riessen et al., 1988; Wirtz, 2014)*.* After feeding the *C. americanus*, beakers were haphazardly placed back into the incubator to minimize environmental effects within incubators.

Chaoborus americanus data collection

Chaoborus americanus were photographed at the start and end of the experiment using a digital camera (Zeiss Axiocam 105, Jena Germany) attached to a dissecting microscope (Zeiss Stemi 508, Jena, Germany). We measured *C. americanus* head capsule length as a proxy for body size (Sæther, 1970) using Zeiss Zen 2.3 software. Measuring insect growth rate can be indicative of insect fitness, as body size is positively correlated with insect fecundity (Harper, 2018). Growth rate was calculated using the following equation (Equation 3-1).

$$growth\ rate = \frac{\left[\frac{(final - initial)}{initial}\right]}{growing\ days}\ (mm/day)$$

Equation 3-1 The growth rate (mm/day) of *C. americanus* was calculated as the *C. americanus* head capsule width at the end of the experiment minus the head capsule width at the beginning of the experiment, divided by the development time (days).

The duration of the long-term experiment was four months (June to August in 2019). The experiment started on June 17th, 2019, which was four weeks after the initial collection day of *D. pulex* and *C. americanus*.

3.3.6 *Chaoborus americanus* **treatment (short-term experiment)**

We conducted an additional experiment in August 2019 to assess the short-term effects of temperature and diet type on *C. americanus* fatty acid profiles and growth rates. Approximately 200 *C. americanus* were collected from cattle tanks at the Experimental Ponds Facility at UBC. Methods for experimental setup, feedings, measurements, and analysis were the same as for the long-term experiment. 120 *C. americanus* of similar-size were each placed into 250ml glass beakers filled with a 50:50 ratio of COMBO (Kilham et al., 1998) and filtered pond water. These 120 beakers remained at ambient temperatures (~20°C) until the start of the experiment three days later, on August 2nd. The *D. pulex* used for feedings were the same *D. pulex* used for feeding *C. americanus* treatments in the long-term experiment. Equation 3-1 was used to calculate the *C. americanus* growth rate.

Apparatus

Per incubator:
Insect larvae
(*Chaoborus americanus*)
= 15 beakers (long-term)
    30 beakers (short-term)

Zooplankton
(*Daphnia pulex*)
= 30 Jars

Phytoplankton
(*Scenedesmus obliquus*)
= 4 bottles

Incubator x 2
Temperature (˚C)
= 12

Incubator x 2
Temperature (˚C)
= 20

Incubator x 2
Temperature (˚C)
= 28

Sample size per temperature treatment

Phytoplankton
(*Scenedesmus obliquus*):
12, 20, 28 ˚C

x 8

Zooplankton
(*Daphnia pulex*)
30 individuals/jar:
12, 20, 28 ˚C

x 60

Insect larvae
(*Chaoborus americanus*)
1 individual/beaker:
12, 20 ˚C

x 30 (long-term
experiment

x60 (short-term
experiment)
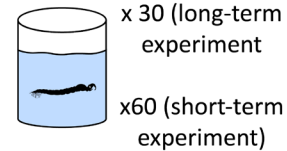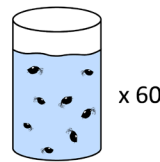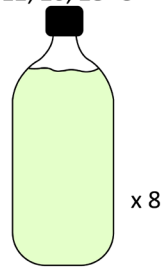
Diet treatments & Feedings

Chaoborus Temperature (°C) (long-term: n = 15 individuals per treatment) (short-term: n = 30 individuals per treatment)	
C12	C20
D12 S12	D12 S12
D20 S20	D20 S20

Treatments = 4

Daphnia Temperature (°C) (n = 16 jars per treatment)		
D12	D20	D28
S12	S12	S12
S20	S20	S20
S28	S28	S28

Treatments = 9

Scenedesmus Temperature (°C) (n = 8 jars per treatment)		
S12	S20	S28

Treatments = 3

Phytoplankton
(*Scenedesmus obliquus*):
12, 20, 28 ˚C

Zooplankton
(*Daphnia pulex*)
30 individuals/jar:
12, 20, 28 ˚C

Insect larvae
(*Chaoborus americanus*)
1 individual/beaker:
12, 20 ˚C
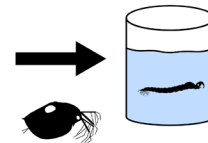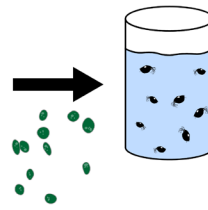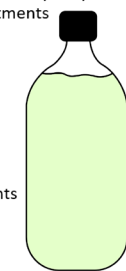
Fed 1×10^6 cells of phytoplankton
per mL of zooplankton COMBO
(x3 per week)

Fed 1 zooplankton
(3x per week)

S = *Scenedesmums obliquus*, D = *Daphnia pulex*, C = *Chaoborus americanus*; numbers 12, 20, and 28 represent the rearing temperature (˚C). From 180 *Daphnia pulex* jars, 60 were designated for each temperature treatment. From these 60, 12 jars from each temperature treatment were used to feed *Chaoborus americanus* and did not contribute to *D. pulex* population counts. The remaining 48 jars per temperature treatment were designated one of three diet types (*Scenedesmus obliquus* reared at 12 ˚C, 20 ˚C, or 28 ˚C), resulting in 16 replicate jars per diet treatment.

Figure 3-1 Illustration of methods for the laboratory experiment involving phytoplankton (*Scenedesmus obliquus*), zooplankton (*Daphnia pulex*), and insect larvae (*Chaoborus americanus*).

3.3.7 Characterization of fatty acids

We used gas chromatography analysis of fatty acid methyl esters (FAME; detailed protocol is described in A.1) to assess fatty acid quantities and composition at the end of the experiment. Fatty acid quantities were standardized by the total fatty acid dry weight for a given sample. For phytoplankton, there were eight samples per temperature treatment collected at the end of the experiment. *D. pulex* populations rapidly declined at the end of the experiment and there was insufficient biomass remaining to accurately assess *D. pulex* fatty acid profiles. The sample sizes at the end of the *C. americanus* long-term and short-term experiment are presented in Table 3-1. We pooled *C. americanus* individuals together to ensure that there would be sufficient biomass to detect a fatty acid signal, and the sample sizes for fatty acid analyses can also be found in Table 3-1.

Technical issues during *C. americanus* FA analyses reduced the sample sizes further. In five of the *C. americanus* tubes the sample evaporated during the methylation process, and in two of the samples, no internal standard signal was detected. These samples were not useable for FA analysis.

Table 3-1 Sample sizes for both the long-term (approximately four months) and short-term (ten days) experiments for *C. americanus* fatty acid analyses.

Treatment	Long-term Experiment			Short-term Experiment		
	n = # Individuals	Sample size sent to lab (n = # vials)	Sample size after processing (n = # vials)	n = # Individuals	Sample size sent to lab (n = # vials)	Sample size after processing (n = # vials)
C12 × D12A12	23	2	2	26	4	3
C12 × D20A20	18	2	2	27	4	3
C20 × D12A12	13	1	0	25	4	4
C20 × D20A20	11	1	0	25	4	1

3.3.8   Statistical analyses

Daphnia pulex population size and Chaoborus americanus growth rate

To examine whether rearing temperature and diet type affected overall *D. pulex* population size, we averaged the population size across census points for each jar. The population sizes from jars of *D. pulex* used to feed *C. americanus* were not counted or used in the planned analysis. Each jar thus contributes one data point. We used a linear mixed-effects model (package lmerTest; Kuznetsova et al., 2017) with 'incubator' modelled as a random factor and 'rearing temperature' and 'diet type' modeled as fixed factors to examine whether there were significant differences in mean population size across treatments.

To test whether rearing temperature and diet type affected *C. americanus* growth rates we used a linear model with the fixed factors of 'rearing temperature' and 'diet type'. Unlike the *D. pulex* model stated above, we did not include the random factor of 'incubator'. *C. americanus* were not sampled from multiple incubators for a given treatment because we designated only one

incubator per treatment, unlike with *D. pulex* treatments that had two designated incubators per treatment.

Fatty acids – Scenedesmus obliquus and Chaoborus americanus

To examine the effects of temperature on fatty acid composition for *S. obliquus* and *C. americanus* (long-term and short-term experiments) we investigated the following fatty acid traits: 1) Total fatty acid quantity per individual dry mass, 2) Total quantities of fatty acids by saturation type (polyunsaturated, monounsaturated, saturated), 3) Total quantities of specific fatty acids of interest (DHA, EPA, ALA, LIN, Oleic, and ARA) and 4) Omega-3:Omega-6 ratio. We used Analysis of Variance (ANOVA) to test 1) Total fatty acid quantity, and 4) Omega-3:6 ratio. We used a linear mixed-effects model for 2) Fatty acid saturation type and 3) Specific fatty acids of interest, with the two models including the random factor of 'vial' as each vial provided multiple measurements. For the omega-3:6 ratio for *S. obliquus* we excluded a single outlier based on the extreme difference between the outlier value and the mean (Benhadi-Marín, 2018).

All statistical analyses were conducted in R version 3.6.2 (R Core Team, 2019).

3.4   Results

3.4.1   Phytoplankton – *Scenedesmus obliquus*

Total fat (µg) per algae dry weight

There was a significant effect of temperature on total fatty acids produced by *S. obliquus* ($F_{(2, 21)}$ = 3.784, p = 0.040), with *S. obliquus* producing the lowest quantities at 20°C ($\bar{x}$ = 0.0072µg, SD = 0.0016) and the highest quantities at 28°C ($\bar{x}$ = 0.0124µg, SD = 0.0047; Figure 3-2).
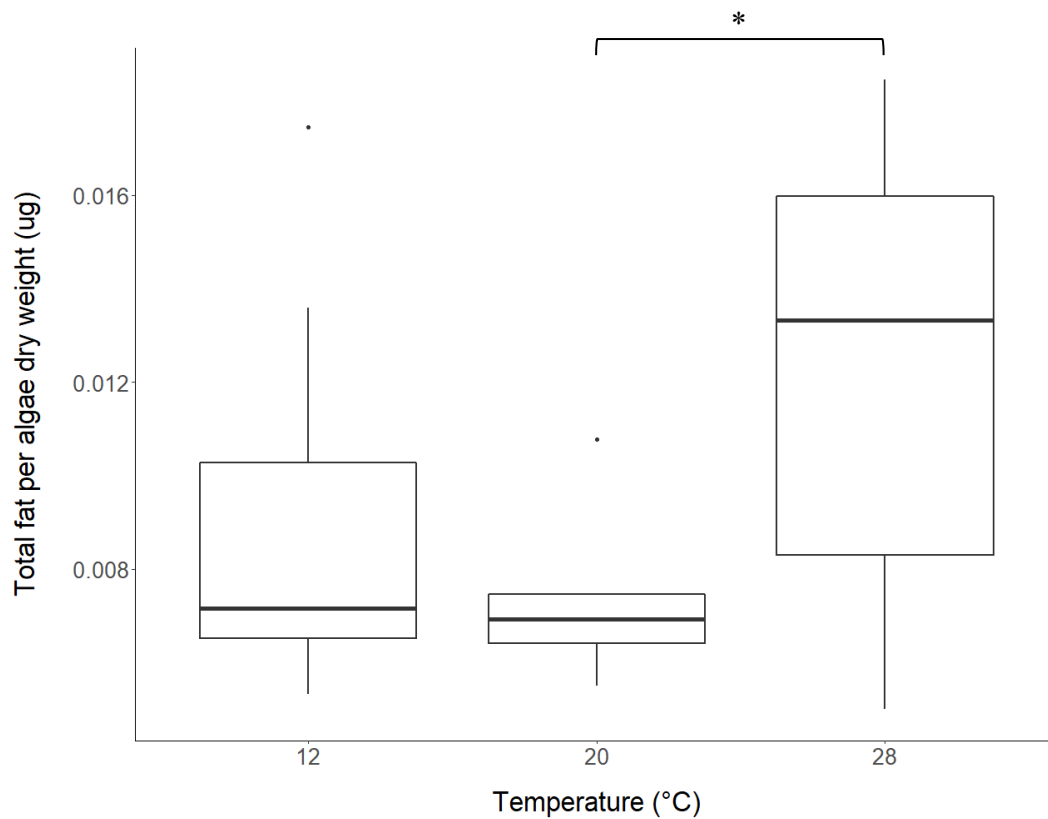
Figure 3-2 The total quantity of fatty acid per unit dry weight (µg) of *Scenedesmus obliquus* at each temperature treatment.; Asterisk indicates statistically significant differences.

Effect of temperature on different FA categories

Scenedesmus obliquus produced different quantities of monounsaturated (MUFA), polyunsaturated (PUFA), and saturated fatty acids (SFA) across temperature treatments (FA

type: $F_{(2, 42)} = 52.02$, $p < 0.0001$). Across the three growing temperatures, *S. obliquus* produced more PUFA than MUFA or SFA acids (average mean across all temperatures: $\bar{x} = 0.0013\mu g$, SD = 0.000813; Figure 3-3). The effect of temperature on total fatty acids produced differed according to fatty acid type (interaction: $F_{(4, 42)} = 4.77$, $p = 0.003$). PUFA quantities increased strongly with temperature, but temperature had weaker effects on MUFA or SFA production (Figure 3-3). There was a non-significant main effect of temperature on these three fatty acid types (temperature: $F_{(2, 21)} = 3.22$, $p = 0.06$).
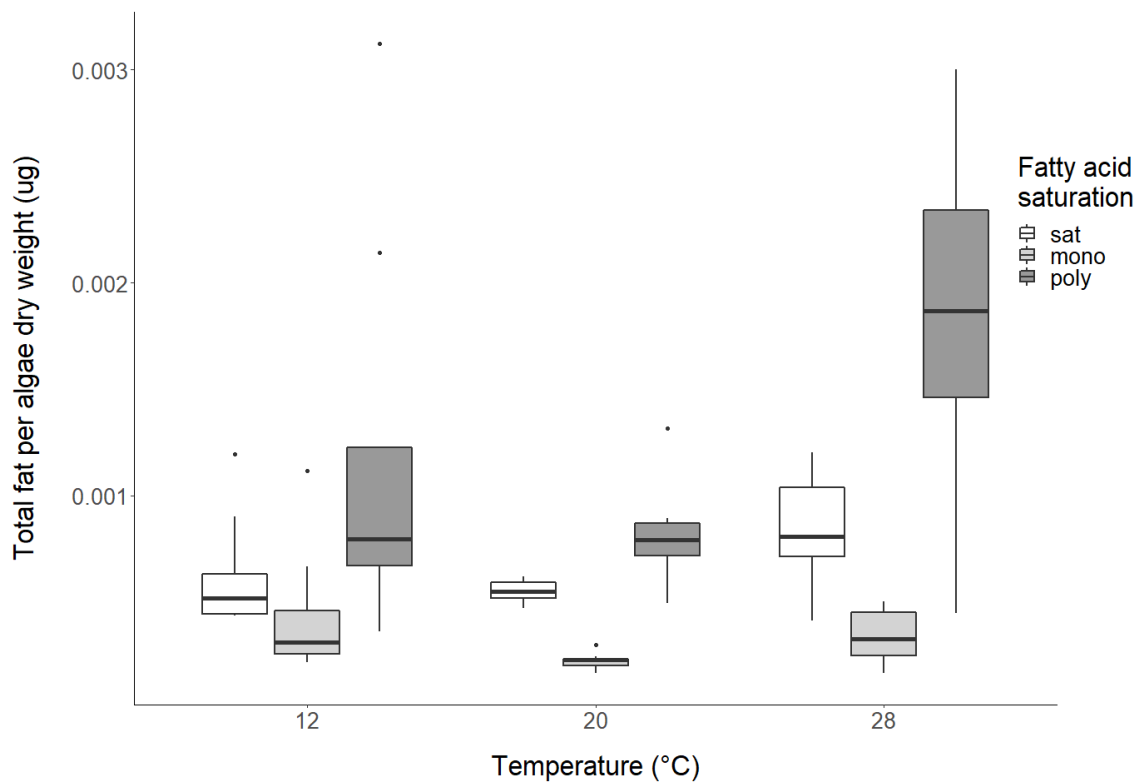
Figure 3-3 The total quantity of fatty acid (μg) for *Scenedesmus obliquus* per type of fatty acid saturation for each temperature treatment. The legend indicates the type of saturation, with sat = saturated fatty acids, mono = monounsaturated, and poly = polyunsaturated.

The effect of temperature on specific fatty acids of interest

EPA and DHA

There was no effect of temperature on the quantities of EPA and DHA produced by *S. obliquus* ($F_{(2, 1.24)}$ = 2.12, p = 0.39; Figure 3-4A). *S. obliquus* made more EPA than DHA ($F_{(1, 1.32)}$ = 99.05, p = 0.033). The interaction between temperature and FA type was not significant, indicating that the effect of temperature did not differ between the two types of fatty acid ($F_{(2, 1.32)}$ = 7.23, p = 0.194).

ALA, LIN, and Oleic acid

The effect of rearing temperature on fatty acid dry weight differed by fatty acid type (temperature × FA type: $F_{(4, 42)}$ = 5.34, p = 0.0014; Figure 3-4B), and there was no main effect of temperature ($F_{(2, 21)}$ = 2.61, p = 0.097). ALA production increased with temperature, but temperature did not strongly affect how much LIN or Oleic Acid was produced by *S. obliquus*. Overall, *S. obliquus* produced more ALA than Oleic or LIN, and *S. obliquus* made slightly more Oleic acid than Linoleic acid, except at 28°C (comparison between fats for a given temperature: $F_{(2, 42)}$ = 70.54, p < 0.0001) (for specific average mean values for all fatty acids, please refer to Table A.2).
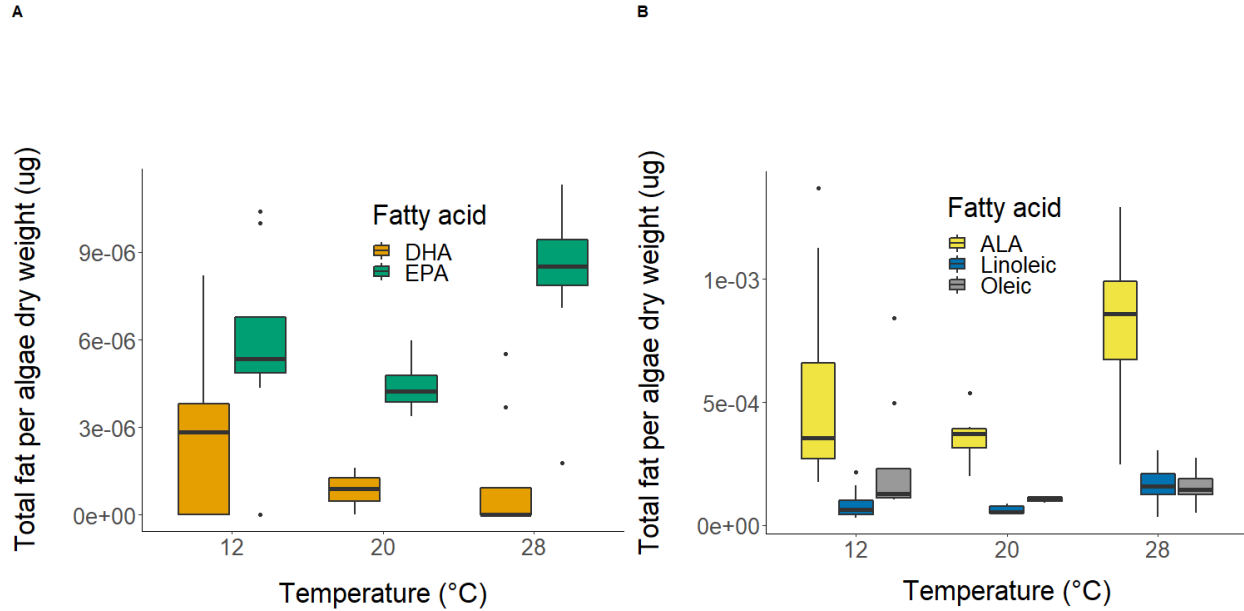
Figure 3-4 The quantities of specific fatty acids of interest produced by *Scenedesmus obliquus* for each temperature treatment. A) The quantities of Eicosapentaenoic (EPA) and Docosahexaenoic (DHA), with DHA being significantly higher for each temperature treatment than EPA but the quantities of both EPA and DHA did not vary with temperature. B) The quantities of α-Linolenic acid (ALA), Linoleic, and Oleic acid did, with ALA having the highest quantities within each temperature treatment.

Omega-3:Omega-6 ratio

The omega-3:omega-6 ratio was highest in the 12 ˚C-reared *S. obliquus* but this effect was not statistically significant ($F_{(2, 20)}$ = 1.869, p = 0.18; Figure 3-5).
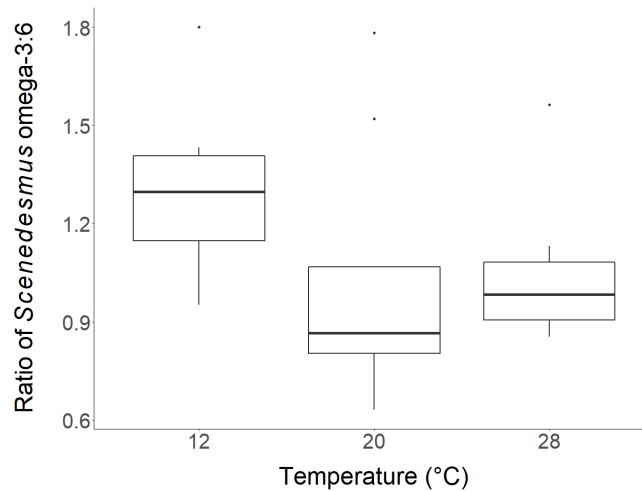
Figure 3-5 The omega-3:omega-6 ratio for *Scenedesmus obliquus* for each temperature treatment.

3.4.2　Zooplankton – *Daphnia pulex*

Daphnia pulex population size

    Daphnia pulex population size was affected by temperature ($F_{(2, 3)}$ = 12.84, p = 0.034; Figure 3-6). Averaged across all census dates, *D. pulex* grown at 28 ˚C maintained approximately half the population size as those reared at 12 ˚C or 20 ˚C.

    The effect of *S. obliquus* diet type on *D. pulex* population size depended on *D. pulex* rearing temperature (interaction: $F_{(4, 131)}$ = 2.48, p = 0.047; Figure 3-6; Table A.3). *D. pulex* fed cold-reared *S. obliquus* had higher average population sizes than *D. pulex* fed 20 ˚C- or 28 ˚C-reared *S. obliquus*, and this effect of diet was strongest when *D. pulex* were also reared at 12 ˚C. For temporal trends in *D. pulex* population size, please see A.4.
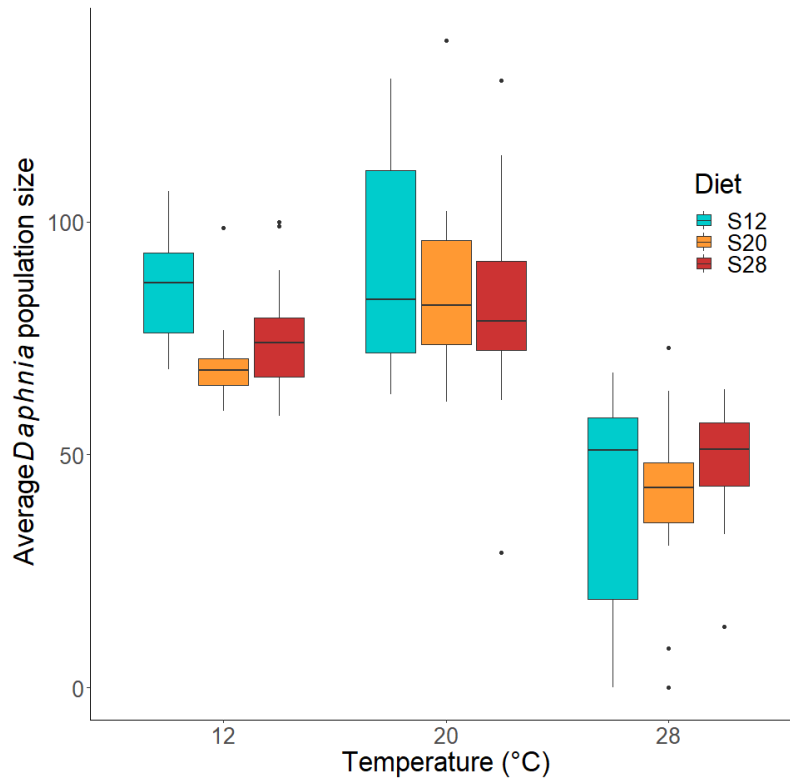
Figure 3-6 *Daphnia pulex* population size averaged over 3 months (with starting populations of 30 individuals occurring in May and first measurements taken place in June). S12, S20, and S28 represents *Scenedesmus obliquus* reared at of 12, 20, and 28°C. *D. pulex* rearing temperature is plotted on the x-axis.

3.4.3   Insect larvae – *Chaoborus americanus*

Chaoborus americanus growth rate

Long-term

There was an effect of temperature on *C. americanus* growth rate ($F_{(1, 61)} = 4.42$, p = 0.04) and the effect of diet type depended on the *C. americanus* rearing temperature (interaction: $F_{(1, 61)} = 4.75$, p = 0.03; Figure 3-7). When reared at 20 ˚C, *C. americanus* growth rate was higher when they were fed cold-reared diet. There was little difference in growth rate between the diet types in the 12 ˚C rearing temperature.

Short-term

In contrast to the long-term experiment, *C. americanus* growth rate was affected by diet type ($F_{(1, 99)} = 9.50$, $p = 0.003$) but not temperature in the short-term experiment ($F_{(1, 99)} = 1.54$, $p = 0.22$; Figure 3-7). There was no interaction between diet type and temperature ($F_{(1, 99)} = 0.20$, $p = 0.66$). *C. americanus* fed cold reared *D. pulex* grew faster than those fed warm-reared *D. pulex*.
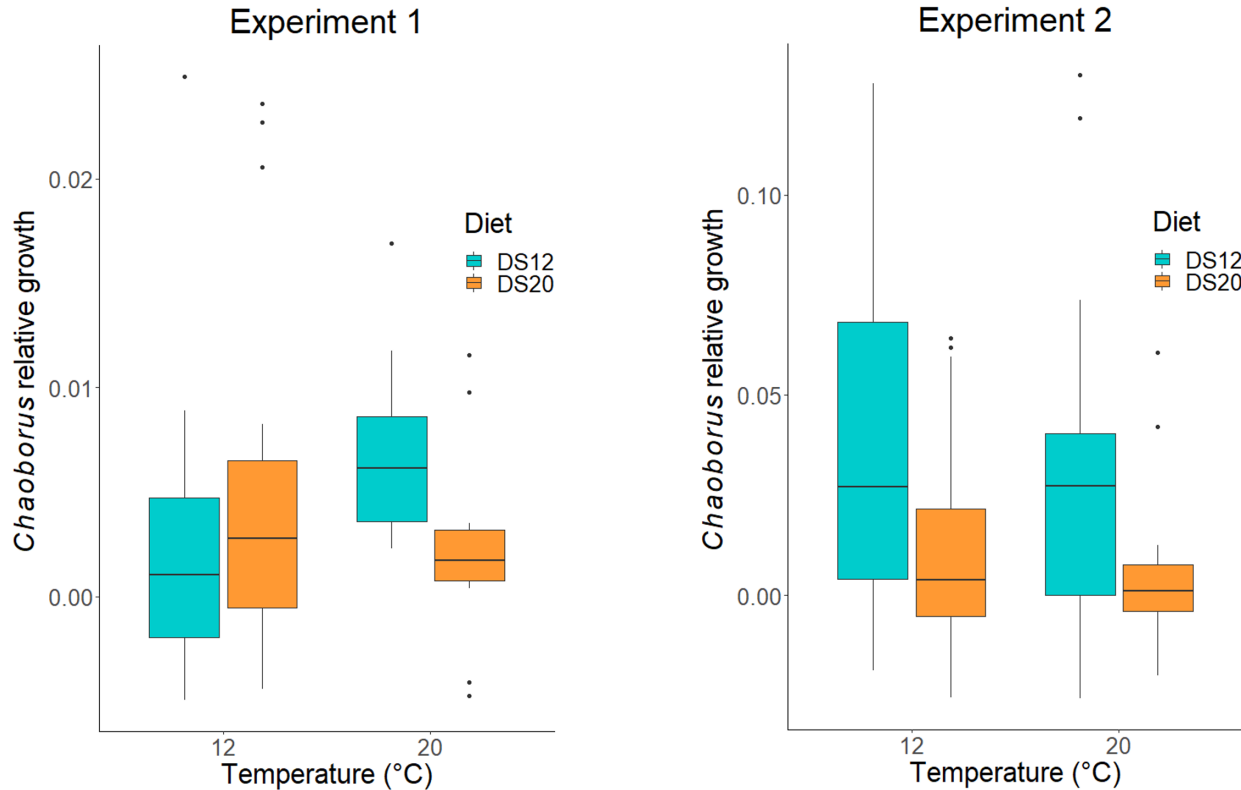
Figure 3-7 Insect larvae, *Chaoborus americanus*, relative growth rate for the long-term experiment (Experiment 1; growth over approximately four months) and short-term experiment (Experiment 2; growth over 10 days). Diet type is presented in the legend, with DS12 and DS20 standing for *Daphnia pulex* fed *Scenedesmus obliquus* and both reared at either 12°C or 20°C.

Chaoborus americanus fatty acids

Due to the loss of samples by the end of the both the long-term and short-term experiments (please refer to Table 3-1 for number of replicates) we could not conduct any statistical tests to examine how temperature affected fatty acid quality in *C. americanus* in the

short-term experiment. Despite the very low sample sizes, we were able to examine: (a) Both experiments: whether diet type affected *C. americanus* fatty acid profiles for larvae reared at 12 ˚C, and (b) Short-term experiment: the effect of growing temperature (12 ˚C vs. 20 ˚C) on *C. americanus* fatty acid profiles. The question of how both diet quality and environmental temperature affects consumer quality could not statistically assessed but will be discussed later based on the results analyses here.

Total fat (µg) per Chaoborus americanus dry weight

For the long-term experiment, there was no apparent effect of diet type on *C. americanus* total fatty acid quantity for *C. americanus* reared at 12 ˚C and fed diet reared at 12 ˚C or 20 ˚C ($F_{(1, 2)} = 0.195$, $p = 0.702$; Figure 3-8; Table A.6). This was also true for the short-term experiment, whereby there was no effect of diet type on *C. americanus* fatty acid quantity for *C. americanus* reared at 12 ˚C ($F_{(1, 3)} = 1.00$, $p = 0.39$). Additionally, for the short-term experiment, there was no effect of temperature on *C. americanus* fed a diet reared at 12 ˚C ($F_{(1, 5)} = 0.032$, $p = 0.86$) (for a full list of mean values and SD, please refer to Table A.5).
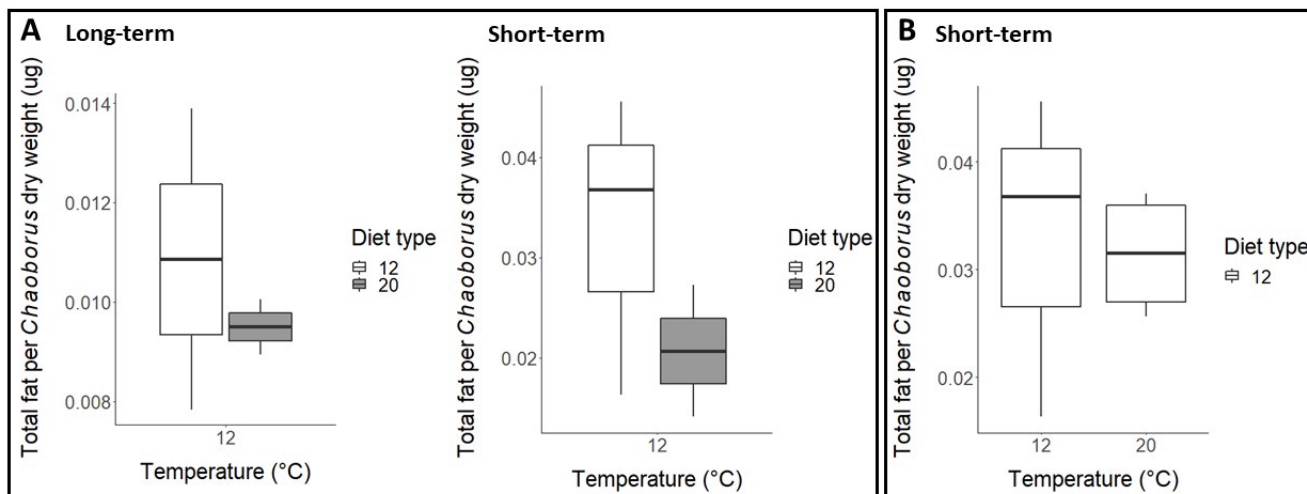
Figure 3-8 The total quantity of fatty acid per *Chaoborus americanus* dry weight (µg) found in *C. americanus* reared under two temperatures and diet types. The legend indicates the diet type fed to *C. americanus*, with 12 = *D. pulex* fed algae, both reared under 12°C, and 20 = *D. pulex* fed algae, both reared under 20°C. A). On the left is the long-term experiment (spanning approximately four months), and on the right is the short-term experiment (spanning 10 days). B) The effect of temperature on saturation quantity for the short-term experiment.

Saturation type

Long-term experiment: effect of diet type

There was no effect of diet type on the quantities of SFAs or UFAs for *Chaoborus* reared at 12°C ($F_{(1, 2)} = 0.195$, $p = 0.702$; Figure 3-9A). *C. americanus* contained similar quantities of the three fatty acid types ($F_{(2, 4)} = 3.71$, $p = 0.123$), and there was no interaction between the type of fatty acid and diet type ($F_{(2, 4)} = 0.002$, $p = 1.00$; for a full list of model outputs, please refer to Table A.6).

Short-term experiment: effect of diet type

Chaoborus americanus contained significantly different quantities of SFAs, MUFAs, and PUFAs irrespective of treatment ($F_{(2, 6)} = 22$, $p < 0.01$; Figure 3-9A), but this effect was primarily observed in *C. americanus* fed 20 ˚C-reared *D. pulex* (FA type x temperature interaction: $F_{(2, 6)} =$

66

28, p < 0.001). Overall higher quantities for each fat type were observed in *C. americanus* fed a cold reared diet compared to a warm reared diet, but the main effect of diet type was not statistically significant ($F_{(1, 3)}$ = 1.00, p = 0.39; for a full list of model outputs, please refer to Table A.6).
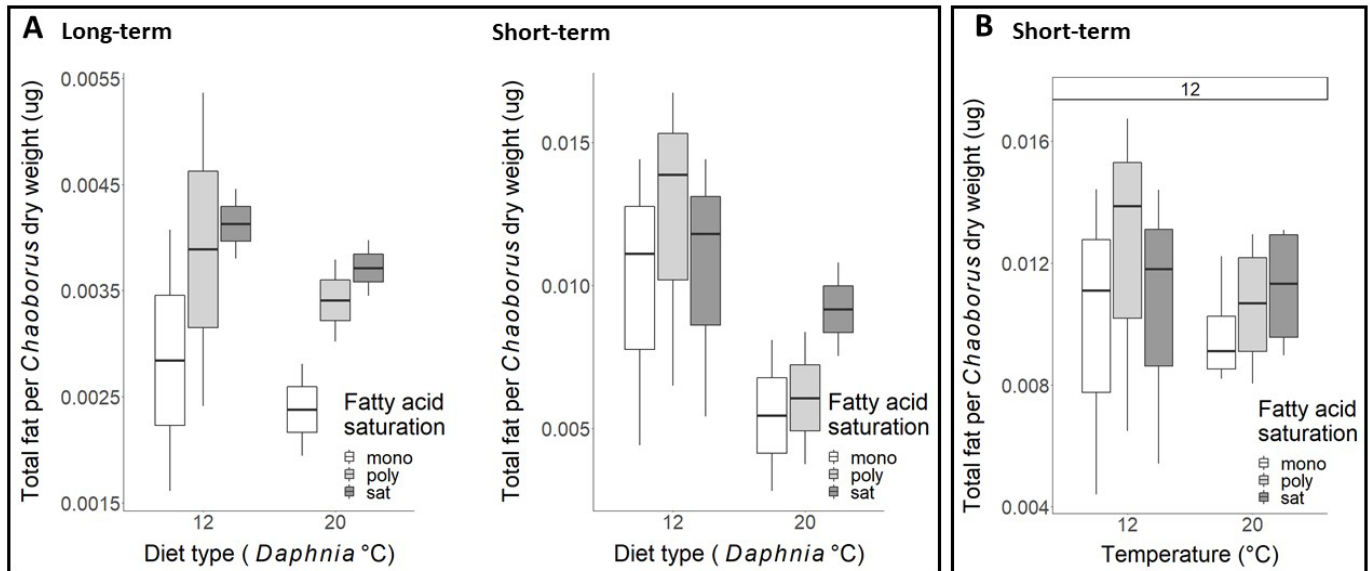
Figure 3-9 *Chaoborus americanus* fatty acid quantity (μg) for a given fatty acid saturation type. The legend presents fatty acid saturation type, with mono = monounsaturated, poly = polyunsaturated, and sat = saturated. A) The effect of diet type on saturation quantity. On the left is the long-term experiment (spanning approximately four months), and on the right is the short-term experiment (spanning 10 days). Presented on the x-axis is the diet type fed to *Chaoborus americanus*, with 12 = *Daphnia pulex* fed algae, both reared under 12°C, and 20 = *Daphnia pulex* fed algae, both reared under 20°C. B) The effect of temperature on saturation quantity for the short-term experiment.

Short-term experiment: effect of rearing temperature

The effect of temperature depended on the type of fatty acid (interaction: $F_{(2, 10)}$ = 4.22, p = 0.047) and there was a significant difference in the quantities of SFAs, MUFAs, and PUFAs within *C. americanus* ($F_{(2, 10)}$ = 8.1, p = 0.008; Figure 3-9B). PUFAs were the most abundant fatty acid type found in *C. americanus* at 12°C ($\bar{x}$ = 0.0124 μg, SD = 0.0053), and the least abundant fatty acid type was MUFAs found in *C. americanus* at 20°C ($\bar{x}$ = 0.0097 μg, SD = 0.0018) (Figure 3-9; for a full list of mean and SD values, please refer to Table A.5; for a full list of model outputs, please refer to Table A.6).

Specific fatty acids of interest

In the long-term experiment, there was no effect of diet type on specific fatty acid quantities ($F_{(1, 2)} = 0.011$, $p = 0.77$); however, *C. americanus* produced different quantities of the six fatty acids analysed here ($F_{(5, 10)} = 25$, $p < 0.0001$; Figure 3-10A). There was no significant interaction between diet type and the type of fatty acid ($F_{(5, 10)} = 0.091$, $p = 0.99$). The results for the short-term experiment were similar. There was no effect of diet type ($F_{(1, 5)} = 2.04$, $p = 0.22$) and the quantities of specific fatty acids were statistically different from one another ($F_{(1, 23)} = 6.71$, $p = 0.016$; Figure 3-10A). There was also no significant interaction between diet type and the type of fatty acid ($F_{(1, 23)} = 0.28$, $p = 0.601$). For both the long and short-term experiment, the fatty acid with the highest concentration was Oleic acid, followed by ALA, then EPA (for a full list of mean and SD values, please refer to Table A.5).

There was no effect of temperature on specific fatty acid quantities in the short-term experiment ($F_{(1, 10)} = 0.002.6$, $p = 0.96$) and there was no interaction between temperature and specific types of fatty acids ($F_{(1, 33)} = 0.27$, $p = 0.61$; Figure 3-10B). The quantities of specific fatty acids were statistically different from one another ($F_{(1, 33)} = 16.89$, $p = 0.000246$), with the highest being Oleic acid and the lowest, DHA.
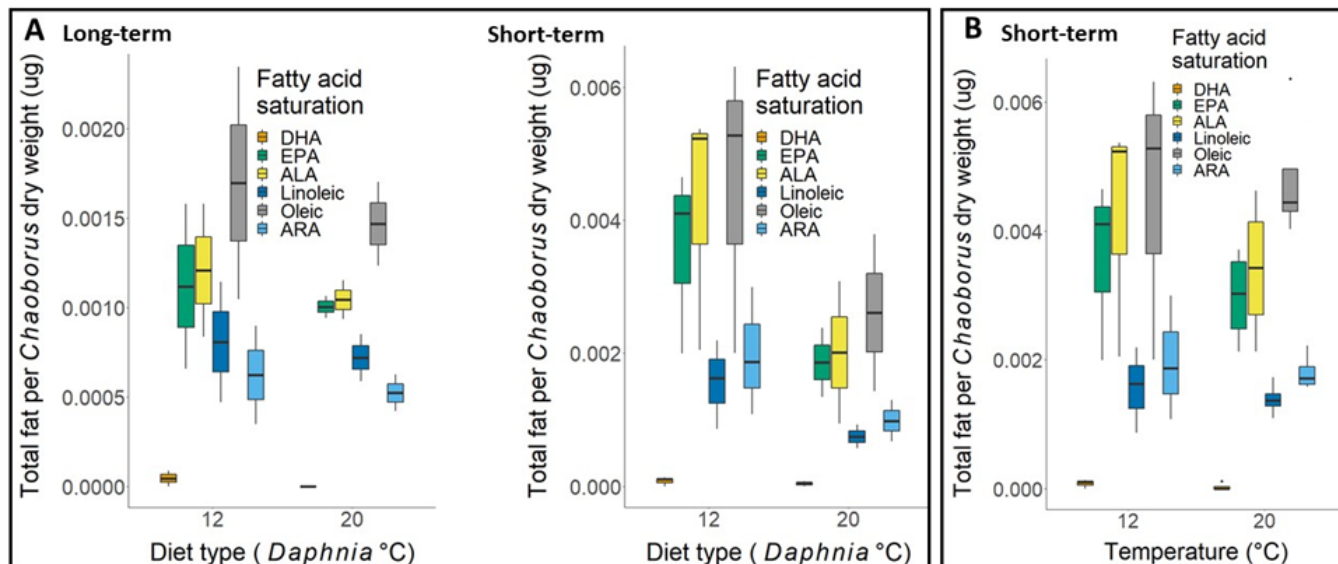
Figure 3-10 *Chaoborus americanus* fatty acid quantity (µg) for fatty acids of specific interest. The legend presents each fatty acid, with ALA = α-Linolenic acid, DHA = Docosahexaenoic, EPA = Eicosapentaenoic, Linoleic = Linoleic acid, Oleic = Oleic acid, and ARA = Arachidonic acid. A) On the left is the long-term experiment (spanning approximately four months), and on the right is the short-term experiment (spanning 10 days). The diet type fed to *Chaoborus americanus* is presented on the x-axis, with 12 = *Daphnia pulex* fed *Scenedesmus obliquus*, both reared under 12°C, and 20 = *Daphnia pulex* fed *Scenedesmus obliquus*, both reared under 20°C. B) The effect of temperature on saturation quantity for the short-term experiment.

Omega-3:Omega-6 ratio

There was no effect of diet type on omega-3:omega-6 ratios in *C. americanus* reared under 12°C in the long-term experiment ($F_{(1, 2)}$ = 0.071, p = 0.82; Figure 3-11A). The same pattern was observed in the short-term experiment ($F_{(1, 3)}$ = 0.012, p = 0.92; Figure 3-11A). There was no effect of temperature on omega-3:omega-6 ratios for *C. americanus* reared at 12°C ($F_{(1, 5)}$ = 0.358, p = 0.58; Figure 3-11B) (for a full list of mean and SD values, please refer to Table A.5).
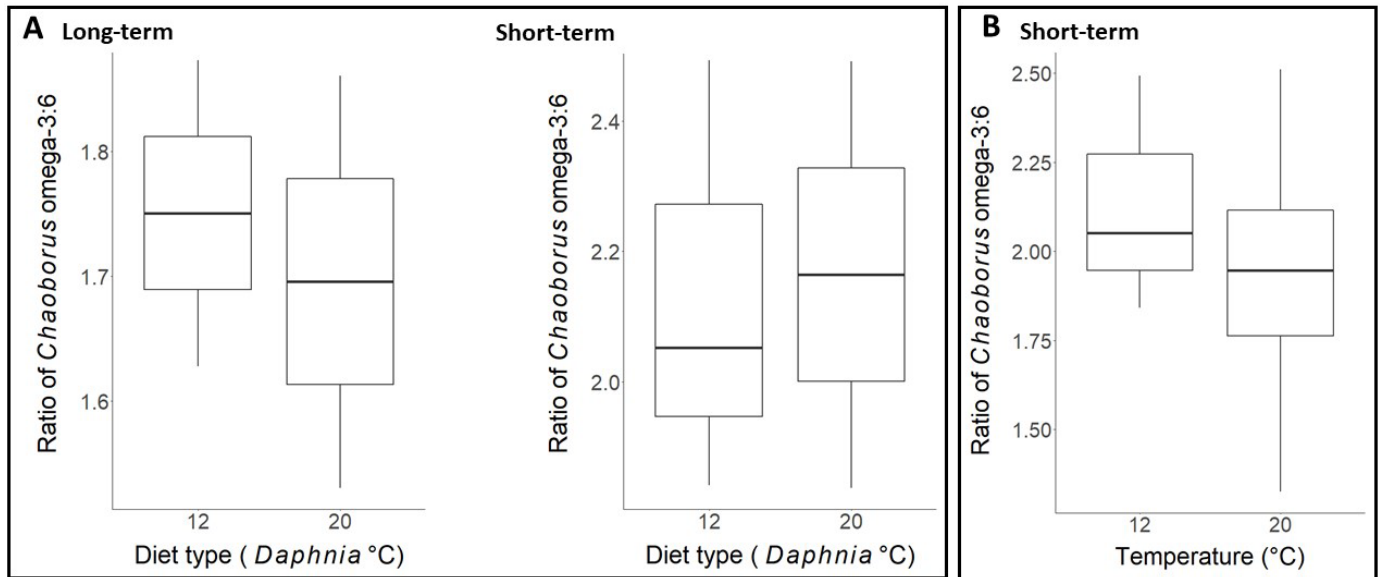
Figure 3-11 The ratio of omega-3:6 for *Chaobours americanus* for each temperature treatment. A) On the left is the long-term experiment (spanning approximately four months), and on the right is the short-term experiment (spanning 10 days). The diet type fed to *Chaoborus americanus* is presented on the x-axis, with 12 = *Daphnia pulex* fed *Scenedesmus obliquus*, both reared under 12°C, and 20 = *Daphnia pulex* fed *Scenedesmus obliquus*, both reared under 20°C. B) The effect of temperature on saturation quantity for the short-term experiment.

3.5   Discussion

The goal of this study was to investigate the hypotheses that (1) temperature affects resource quality on multiple trophic levels, and (2) the nutritional requirements of consumers change with environmental temperature. We used fatty acid (FA) quantity and composition as a measure of resource quality because of their importance for proper growth and development. To address hypothesis (1), we predicted warming to decrease healthy unsaturated fatty acids (e.g., LC-PUFA) and increase unhealthy saturated fatty acids in *S. obliquus*, *D. pulex*, and *C. americanus*. To address hypothesis (2), we made several predictions: a) Warming and warm-reared (nutrient poor) diets will decrease *D. pulex* population size, while warming will increase *C. americanus* growth rates but a warm-reared (nutrient poor) diet will decrease *C. americanus* growth rates, and c) the environmental temperature of the consumer will not change the effects of diet quality. We found that with warming, total *S. obliquus* fatty acid content increased with temperature, and fatty acid composition did not significantly change with warming. Diet type had a strong effect on *D. pulex* population size, but only in the cold rearing temperature. There were no strong effects of rearing temperature on *C. americanus* growth rates, but there were slight effects of diet type. There were no strong effects of rearing temperature or diet type on *C. americanus* fatty acids. Overall, temperature affected algae fatty acid composition and we saw cascading effects of these changes; however, these cascading effects became weaker at higher trophic levels.

The observed trend of increased PUFA production at higher temperatures in *Scendesmus* contrasts with the current literature. Previous studies on phytoplankton found that warming temperatures cause a shift from UFAs (MUFAs and PUFAs) to SFAs and a shift from omega-3 LC-PUFA to omega-6 LC-PUFA (Hixson and Arts, 2016; O'Donnell et al., 2019). Specifically,

71

studies in *S. obliquus* found that warming caused *S. obliquus* to produce fewer FAs, fewer

omega-3 LC-PUFA (e.g., EPA, DHA, and ALA), and more less-nutritious fatty acids (e.g.,

SFAs) (Breuer et al., 2013; Hodaifa et al., 2010; Sikora et al., 2014; Vigeolas et al., 2012). Our

results contrast with some of these findings because at warmer temperatures (specifically 28°C in

our experiment) we observed an increase in total FAs and an increase in PUFAs instead of SFAs.

We also did not observe a decrease in *S. obliquus* omega-3 LC-PUFA (ie. EPA, DHA, and ALA)

with warming, and we had statistically similar ratios of omega-3 LC-PUFA and omega-6 LC-

PUFA at all temperatures. However, we did observe a trend that is in the direction predicted,

with higher omega-3 LC-PUFA at the coldest rearing temperature. It is unclear as to why our

results contrast that of prior studies, with Fuschino et al. (2011) who reared *S. obliquus* under 20

and 28°C having decreased FAs. Fuschino et al. (2011) also found decreased omega-3 LC-

PUFAs (specifically 18-carbon PUFAs), increased SFAs and decreased ALA with warming.

Hodaifa et al. (2010) who also investigated temperature effects on *S. obliquus* fatty acids had

similar results to Fuschino et al. (2011), suggesting that the overall quality of *S. obliquus* should

decrease with warming. It may be possible that an extraneous factor impacted our *S. obliquus*

fatty acid composition, with such variables as variations in starvation levels for algae, fungi, and

copepods impacting the reallocation of FAs within the body and thus FA composition with

temperature (Hemme et al., 2014; Légeret et al., 2016; Péter et al., 2017; Werbrouck et al.,

2016).

Our results for *D. pulex* population size are in accordance with the literature. With

warming we observed a decrease in *D. pulex* population size (only present at the warmest

temperature of 28°C) and the effect of diet depended on the rearing temperature following the

prediction that *D. pulex* fed a cold reared diet had increased population size (especially at our

72

coldest temperature of 12°C). This aligns with the findings from Tseng and O'Connor (2015) and Tseng et al. (2019) who also cultured *D. pulex* in the lab and found that at warmer temperatures population size decreased. There is some discrepancy in the literature where Adamczuk (2020) found *Daphnia magna* subject to a cold and warm treatment to have the highest population size at the warmest treatment; however the treatments conducted by Adamczuk (2020) involved testing a much lower temperature (comparing treatments of 5°C and 25°C). The temperatures from Adamczuk (2020) alongside the temperatures from Tseng and O'Connor (2015), Tseng et al. (2019), and our study suggest that the relationship between temperature and population size for *D. pulex* is non-linear, with an ideal temperature closer to 25°C. The results from diet effects are also in accordance with the literature. With warming we would expect increased metabolic demands by consumers to maintain fitness (Dijkstra et al., 2012; Lemoine and Shantz, 2016) and result in an increased need for a high-quality diet (Boersma et al., 2016; Lemoine et al., 2013). Thus, we would expect an effect of rearing temperature and effect of diet type and we did observe these effects with the highest fitness (i.e., population size) for *D. pulex* fed a cold reared diet (i.e., potentially nutrient rich based on increased omega-3:omega-6 ratio in predicted direction), specifically at the coldest rearing temperature (observed to have a high average population size). However, a result that goes against our predictions is that *D. pulex* did not always have the highest population size when provided a cold reared diet, regardless of *D. pulex* rearing temperature. This result was also observed by von Elert and Fink (2018) who reared *Daphnia magna* and their algal diet at 15, 20, and 25°C and found the fitness measure of somatic growth to be highest for *D. magna* fed an algal diet reared at the same *D. magna* rearing temperature.

The effects of temperature and diet type on *C. americanus* growth rate and FA composition were challenging to determine due to low FA sample sizes. However, the comparisons made on 1) the effects of temperature and 2) the effects of diet type on *C. americanus* growth and FA composition can still be discussed with the understanding that low sample size reduces statistical power and reduces the detection of a statistically significant result (Anderson et al., 2017; de Winter, 2013). With warming we did observe an increase in *C. americanus* growth rate, specifically for *C. americanus* fed a cold-reared (i.e., potentially nutrient rich based on trends in the predicted direction for increased omega-3:omega-6 ratios) diet and reared under 20°C. These results align with the findings that warmer temperatures accelerate the growth of early-instar *Chaoborus* (Frouz et al., 2002; Hanazato and Yasuno, 1989), and that *Chaoborus* can assimilate fatty acids from their diet and show increased growth under a nutrient rich diet (Hixson et al., 2015; Torres-Ruiz et al., 2007). In slight contrast to the literature are the findings from *C. americanus* FA composition. There were no strong effects of rearing temperature and diet type on *C. americanus* FAs and our findings suggest that cascading effects became weaker at higher trophic levels. Findings from Hixson et al. (2015) and Torres-Ruiz et al. (2007) suggest high FA assimilation between phytoplankton and zooplankton, and between zooplankton to aquatic insects, meaning that the FA composition should be fairly similar to that of their diet. The weakening cascading effects in our study system could be in part due to the low FA samples used for analyses, and further investigation with a larger sample size could show stronger cascading effects.

In conclusion, our study presents how temperature and diet quality can impact a simple aquatic community and help make predictions on the quality of organisms with ongoing climate warming. Our data suggest that warming could increase organism quality with increased quantity

of FAs and potentially increased LC-PUFAs (e.g., ALA) important for proper development and growth of organisms. However, our results contrast that of the existing literature that suggests warming could decrease organism quality through decreased fats, such as essential omega-3 LC-PUFA (e.g., EPA, DHA, and ALA). Overall, our findings suggest that consumers may adapt to available resources in their environment, even with potential fluctuations in resource quality. Understanding organism response to resource availability is an important step towards understanding the effects of warming on higher trophic levels. Specifically, further investigation is needed on the underlying mechanisms behind why organism quality may change with warming and the magnitude to which resource quality can affect trophic levels in aquatic systems and beyond into terrestrial systems.

Chapter 4: Conclusion

4.1 Main findings and Discussion

From the meta-analysis we found that temperature did not strongly affect organism body size, but predator presence did strongly affect organism body size. From the lab experiment we found that temperature affected resource quality and changed the nutritional needs of consumers.

There are few studies that directly compare the effects of warming and predator presence on prey nutritional quality, with my meta-analysis being the first synthesis testing the hypotheses that, (1) warming and predator presence affect prey body size (correlated with prey quality), and (2) temperature and predator presence differ in their effects on prey body size. The studies used in my meta-analysis varied in their findings for significant effects of warming, predator presence, and their interaction; however, there was a larger number of studies that found a significant effect of warming (Cavalheri et al., 2019; Gilbert, 2011; Grigaltchik et al., 2016; MacLennan and Vinebrooke, 2016; Pavón-Meza et al., 2007; Peckarsky et al., 2001; Scherer et al., 2013; Weetman and Atkinson, 2004, 2002; Zhao et al., 2014) and predator presence (Costa and Kishida, 2015; Gilbert, 2011; Grigaltchik et al., 2016; Huss et al., 2010; MacLennan and Vinebrooke, 2016; Pavón-Meza et al., 2007; Scherer et al., 2013; Tseng and O'Connor, 2015; Weetman and Atkinson, 2002; Zhao et al., 2014) on prey body size. Most studies found warming to significantly decrease prey body size while the effect of predator presence varied, with generally equal occurrences of predator presence significantly decreasing or increasing prey body size. The interaction between warming and predator presence was not often tested, but the few papers that did test for the interaction had contrasting results (Cavalheri et al., 2019; Costa and Kishida, 2015; Grigaltchik et al., 2016; MacLennan and Vinebrooke, 2016; Pavón-Meza et al., 2007; Tseng and O'Connor, 2015; Weetman and Atkinson, 2004, 2002; Zhao et al., 2014).

Our lab experiment is one of the first studies to assess changes in organism nutritional quality on a multi-trophic scale. We tested the hypotheses that, (1) warming affects resource quality in a simple aquatic community (phytoplankton, zooplankton, insect larvae), and that (2) resource quality will have cascading effects and impact consumer fitness. Our findings support these two hypotheses, but some of our results contrast that of the literature. (1) There are a high number of studies that found warming to decrease total FA quantity (Fuschino et al., 2011; Hodaifa et al., 2010) and shift FA composition to reflect a decreased presence of healthy FAs within an organism (Breuer et al., 2013; Fuschino et al., 2011; Guschina and Harwood, 2009; Vigeolas et al., 2012). We found contrasting results, with warming significantly increasing total FA quantities in the primary producer, *Scenedesmus obliquus,* and had little to no effect on shifting *S. obliquus* FA composition. However, we did observe trends in the predicted direction for FA composition, whereby warming increased healthy omega-3 compared to omega-6 PUFAs (Breuer et al., 2013; Hixson and Arts, 2016; Vigeolas et al., 2012). (2) Many studies found warming to decrease population size (Tseng et al., 2019; Tseng and O'Connor, 2015) and increase growth rates (Frouz et al., 2002; Hanazato and Yasuno, 1989) which was observed for the population sizes of our primary consumer, *Daphnia pulex*, and growth rates of our secondary consumer, *Chaoborus americanus*. We also observed cascading effects of diet quality on consumer population size and growth rates (correlated with fitness), although cascading effects became weaker at higher trophic levels. A cold reared diet (i.e., nutrient rich diet based on the trending higher quantities of omega-3 PUFAs) increased consumer population size and growth rates as predicted (Hixson et al., 2015; Sikora et al., 2014; Torres-Ruiz et al., 2007), but only at a cold rearing temperature for consumers.

The results from my meta-analysis provide general insight into the effects of warming and predator presence on prey quality. My meta-analysis investigated if prey quality will increase or decrease and to what magnitude when exposed to warming and predators, but my meta-analysis did not provide information on how nutrient *composition* may change with warming and predator presence. The findings from my laboratory experiment helped to address how nutrient composition may change with warming, but it did not investigate the effects of predator presence. Integrating the findings from my meta-analysis and laboratory experiment allow me to achieve a more complete understanding of the combined effects of warming and predator presence on prey nutritional quality. Specifically, I can address the knowledge gap on how predator presence could change prey nutrient *composition*. Throughout my discussion it should be made clear that my meta-analysis and lab experiment only investigated aquatic species and so my speculations are most applicable to an aquatic system.

Throughout my thesis I define quality as the quantity and composition of nutrients within an organism's body. The expected increase in prey body size with predator presence suggests that organism nutrient quantity (i.e., mass of specific nutrients, such as fatty acids) would increase. My laboratory experiment also found an increase in prey nutrient quantity, but this was caused by the presence of warming. It is possible that warming and predator presence affect prey organism quality in a similar way, with each factor increasing nutrient quantity within prey. If warming and predators are both present, there is evidence to suggest that warming can increase predator consumption rates (Atkinson, 1994) and potentially accentuate predator effects on prey nutrient quantity through increases in prey body size. This means that prey exposed to both predators and warming are of higher quality compared to prey that live without predators or do not reside in a warm environment.

The response of prey nutritional composition to predator presence is not well understood as there are few studies that have directly tested the effects of predator presence on the composition of specific nutrients within prey. Comparing the results of my meta-analysis and lab experiment, I found both warming and predator presence to share the relationship of increasing prey nutrient quantity when prey are exposed to these two factors. When prey are exposed to warming and predators separately, I found predators to have a stronger effect on prey nutrient quantity. If warming and predator presence have similar effects on prey nutrient quantity, then maybe warming and predator presence share a relationship for how these two factors affect prey nutrient composition. There is evidence to suggest that *D. pulex* exposed to *Chaoborus* kairomones had increased FA allocation to reproduction (Klintworth and Von Elert, 2019). Specifically, Klintworth and Von Elert (2019) found mother *D. pulex* to retain EPA and have higher quantities of EPA compared to mother *D. pulex* not exposed to *Chaoborus* kairomones. This finding suggests that prey nutritional composition may shift to include higher quantities of healthy fatty acids (such as EPA) when prey are exposed to predators. Our lab experiment supports that predator presence and warming may share an effect of increasing prey nutritional quality, as we found a trending increase in quantities of phytoplankton EPA with warming. Thus, predator presence may not only increase nutritional quantity but also shift prey nutritional composition to a healthier composition that provides benefits to all consumers.

There are limitations to integrating the findings from my meta-analysis and laboratory experiment to address how the combined effects of warming and predator presence will affect prey quality. My meta-analysis investigates predator-prey interactions and not multi-trophic interactions in aquatic systems, and it may be possible that the relationship between primary resources and primary consumers can be different than that of primary consumers and secondary

consumers. For example, the relationship between primary and secondary consumers can be affected by resource availability (Klintworth and Von Elert, 2019). If resource availability decreases with temperature, could we expect prey to have a stronger response to predators when in search of food? And if prey have a stronger response to predators, is the response rate the same for prey subject to warming? Klintworth and Von Elert (2019) not only investigated the response of *D. pulex* to predator kairomones, but also the response of *D. pulex* to decreased resource availability. Under food limited environments and predator kairomones, *D. pulex* experienced that greatest shift in FA composition, with higher retention of healthy EPA allocated towards reproduction.

Other limitations to my meta-analysis and laboratory experiment are that they did not investigate the effects of warming on community level changes in prey nutritional quality or the effects of exposure time of prey to warming and predator presence (i.e., plastic vs evolutionary response) that have documented effects on prey nutritional quality. Community level changes could outweigh species level changes if prey community composition is more sensitive to the factors of warming and predator presence. For example, warming can affect the availability of high-quality species within communities by shifting community composition from larger-bodied species to smaller-bodied species through size-selective predation (MacLennan et al., 2015). Specifically, size-selective predation can remove larger-bodied species from prey populations and leave behind smaller-bodied prey species (MacLennan et al., 2015). If prey community composition is more sensitive to the factors of warming and predator presence, then the effects of warming and predator presence on the individual level may not be large enough to exchange the roles of high and low quality species.

The response of prey nutritional quality to warming and predator presence could also change based on the time of exposure to warming and predator presence. With short-term effects we can observe a within generation response to warming and predators (i.e., phenotypically plastic response) compared to long-term effects where we observe between generation responses to these two factors (i.e., evolutionary response). For example, looking at prey body size, a plastic response of prey body size to warming and predator presence could be the removal of large individuals from the population, leaving behind only small individuals (Atkinson and Sibly, 1997). An evolutionary response of prey body size could be that individuals reproduce at a younger age and grow to a smaller size because of multiple generations having undergone natural selection (i.e., evolution in life history strategies) (Blanckenhorn, 2000).

4.2   Implications of this research

The stronger effect of predator presence than warming on prey body size (correlated with organism quality) indicates that greater care must be taken when interpreting the results of studies that assess the effects of temperature on organism body size in the absence of biotic factors (e.g., predator presence). The effects of predator presence on organism body size may outweigh those of temperature, even with ongoing global warming. Additionally, although my thesis focused on the effects of warming and predator presence on aquatic organisms, these findings could have important implications for terrestrial systems. Aquatic and terrestrial food webs provide each other with large quantities of the key elements (carbon, nitrogen, and phosphorus) that make up important nutrients (Elser et al., 2000; Wallace et al., 1997). For example, amphibians and insects are sources of detritus material, which provides carbon and nitrogen for terrestrial plants (Gratton et al., 2008). Additionally, aquatic insects can supply

essential fatty acids to birds, such as healthy omega-3 polyunsaturated fatty acids (Twining et al., 2018). For example, tree swallow chick survival decreased when the biomass of more nutritious aquatic insects decreased and the biomass of less nutritious terrestrial insects increased (Twining et al., 2018).

4.3   Recommendations for future research

My thesis used fatty acids and body size as proxies for organism quality. It is possible that other nutrients, such as carbohydrates and proteins, may respond differently to temperature, and that body size may not be a suitable indicator of quality. For example, does the relationship between size and quality change between animals and plants? Further studies testing the relationship between body size and quality are needed to understand variation in quality between different types of organisms. These studies could then be compiled in a future meta-analysis to synthesize and compare our understanding of variation in quality between species and the potential patterns that arise for organism quality. Another limitation of my study was the finite quantity of food available to consumers that does not mimic natural nutrient fluctuations. Such events as algal blooms would impact resource availability and potentially alter predator-prey interactions. Further research is needed into how fluctuations in resource availability could alter predator-prey interactions and prey nutritional quality.

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Appendices

Appendix A - Testing the effects of warming and diet type on fatty acids and consumer fitness

A.1   Protocol for Gas chromatography (GC) analysis of fatty acid methyl esters (FAME). Text is taken from Tseng, Di Filippo et al manuscript currently in review.

Gas chromatography (GC) analysis of fatty acid methyl esters (FAMEs) was performed at the end of all experiments. A 70mL sample of each replicate culture bottle was filtered onto a 25mm glass microfiber filter (GE Healthcare Life Sciences Whatman). To each sample, 2 mL of 3 M methanolic HCL (Sigma-Aldrich) and 0.5mL of hexane (Sigma-Aldrich) were added before overnight incubation at 80°C. To the cooled sample, 2mL of 0.9% saline and 1.5mL of hexane was added and the mixture was vortexed. After separation, the upper solvent layer was transferred to a 2 mL vial and analyzed using a GC (Scion Instruments Canada, Edmonton, Alberta, Canada). Hydrogen gas was used as a carrier through a 50m column (Agilent J&W CP-Sil 88 for FAME; Santa Clara, California, USA) and the sample was detected using a flame ionization detector. Peak identification used known standards (mostly GLc455 and GLc37, NuChek Prep Inc., Elyysian Minnesota, USA). Fatty acid quantification was based on the relative peak area between individual fatty acids and the internal standard (C19:0, 0.5mg/sample).

A.2 The average quantities (ug) of fatty acids for *Scenedesmus obliquus* in each temperature treatment for types of saturation, fatty acids of interest, and omega-3 and omega-6 polyunsaturated fatty acids.

Temp (°C)	Fat total	Saturation	Fatty Acid	Omega	$\bar{x}$ (ug)	SD
12	Total				9.02×10^{-3}	4.31×10^{-3}
		MUFAs			4.41×10^{-4}	3.09×10^{-4}
		PUFAs			1.18×10^{-4}	9.47×10^{-4}
		SFAs			6.25×10^{-4}	2.76×10^{-4}
			ALA		5.46×10^{-4}	4.51×10^{-4}
			DHA		2.75×10^{-6}	2.85×10^{-6}
			EPA		5.77×10^{-6}	3.29×10^{-6}
			LIN		5.77×10^{-5}	6.57×10^{-5}
			Oleic		2.57×10^{-4}	2.70×10^{-4}
				Omega-3	6.68×10^{-4}	5.12×10^{-4}
				Omega-6	5.53×10^{-4}	5.16×10^{-4}
				Omega-3: Omega-6	1.31	0.281
20	Total				7.20×10^{-3}	1.60×10^{-3}
		MUFAs			2.24×10^{-4}	4.04×10^{-5}
		PUFAs			8.22×10^{-4}	2.36×10^{-4}
		SFAs			5.51×10^{-4}	5.26×10^{-5}
			ALA		3.60×10^{-4}	9.75×10^{-5}
			DHA		8.43×10^{-7}	6.22×10^{-7}
			EPA		4.40×10^{-6}	8.77×10^{-7}
			LIN		6.25×10^{-5}	1.70×10^{-5}
			Oleic		1.05×10^{-4}	1.05×10^{-5}
				Omega-3	3.99×10^{-4}	1.18×10^{-4}
				Omega-6	4.23×10^{-5}	1.50×10^{-5}
				Omega-3: Omega-6	1.02	0.406
28	Total				1.24×10^{-2}	4.74×10^{-3}
		MUFAs			3.42×10^{-4}	1.29×10^{-4}
		PUFAs			1.82×10^{-4}	7.99×10^{-4}
		SFAs			8.38×10^{-4}	2.53×10^{-4}
			ALA		8.10×10^{-4}	3.32×10^{-4}
			DHA		1.15×10^{-6}	2.18×10^{-6}
			EPA		8.05×10^{-6}	2.82×10^{-6}
			LIN		1.64×10^{-4}	8.66×10^{-5}
			Oleic		1.59×10^{-4}	7.00×10^{-5}
				Omega-3	8.99×10^{-4}	3.69×10^{-4}
				Omega-6	9.91×10^{-4}	4.35×10^{-4}
				Omega-3: Omega-6	1.05	0.229

A.3 Results from a linear mixed-effects model (Type III analysis) for *Daphnia pulex* population size with 'incubator' modelled as a random factor and 'rearing temperature' and 'diet type' modeled as fixed factors to examine whether there were significant differences in mean population size across treatments.

	df	Sum Sq.	Test Mean Sq.	F	p
Diet	2	1206.3	603.2	2.0707	0.13019
Rearing Temperature	2	7479.5	3739.7	12.8392	**0.03382***
Diet x Rearing Temp.	4	2892.2	723.0	2.4823	**0.04687***

*p<0.5

A.4   Results from mixed effects models and Type III ANOVA for zooplankton population size for all months and each month separately. All models included the fixed effects of diet type, rearing temperature, and their interactions. The random effect included incubator number. For the model looking at all months together, month was included as a fixed effect as well as the interactions between month and diet type and month and rearing temperature, and jar number was included as a random effect.

For the first measurements conducted in June, *Daphnia* fed algae reared at the coldest temperature had relatively similar population sizes compared to *Daphnia* fed algae reared at ambient and warm temperatures. Only *Daphnia* rearing temperature had a significant effect on *Daphnia* population size ($F_{2, 134}$; $p < 0.001$). For July, there was no significant effect of *Daphnia* temperature, diet type, or their interaction but 23% of the variance is explained by the random effect of incubator (incubator variance = 361.6, SD = 19.02; Residual variance = 1546.4, SD = 39.32). For August, there was a significant effect for both *Daphnia* temperature ($F_{(2, 3)} = 99.14$, $p < 0.001$) , diet type ($F_{(2, 131)} = 12.56$, $p < 0.01$), and their interaction $F_{(4, 131)} = 6.27$, $p < 0.001$. After two months of growth, *Daphnia* population size appeared the highest, occurring in the month of July ($\bar{x} = 120$ *Daphnia*, SD = 47.1). After the fourth and final month (August) *Daphnia* showed a large decline and had the lowest average population size compared to prior months (August: $\bar{x} = 31$, SD = 20.90).
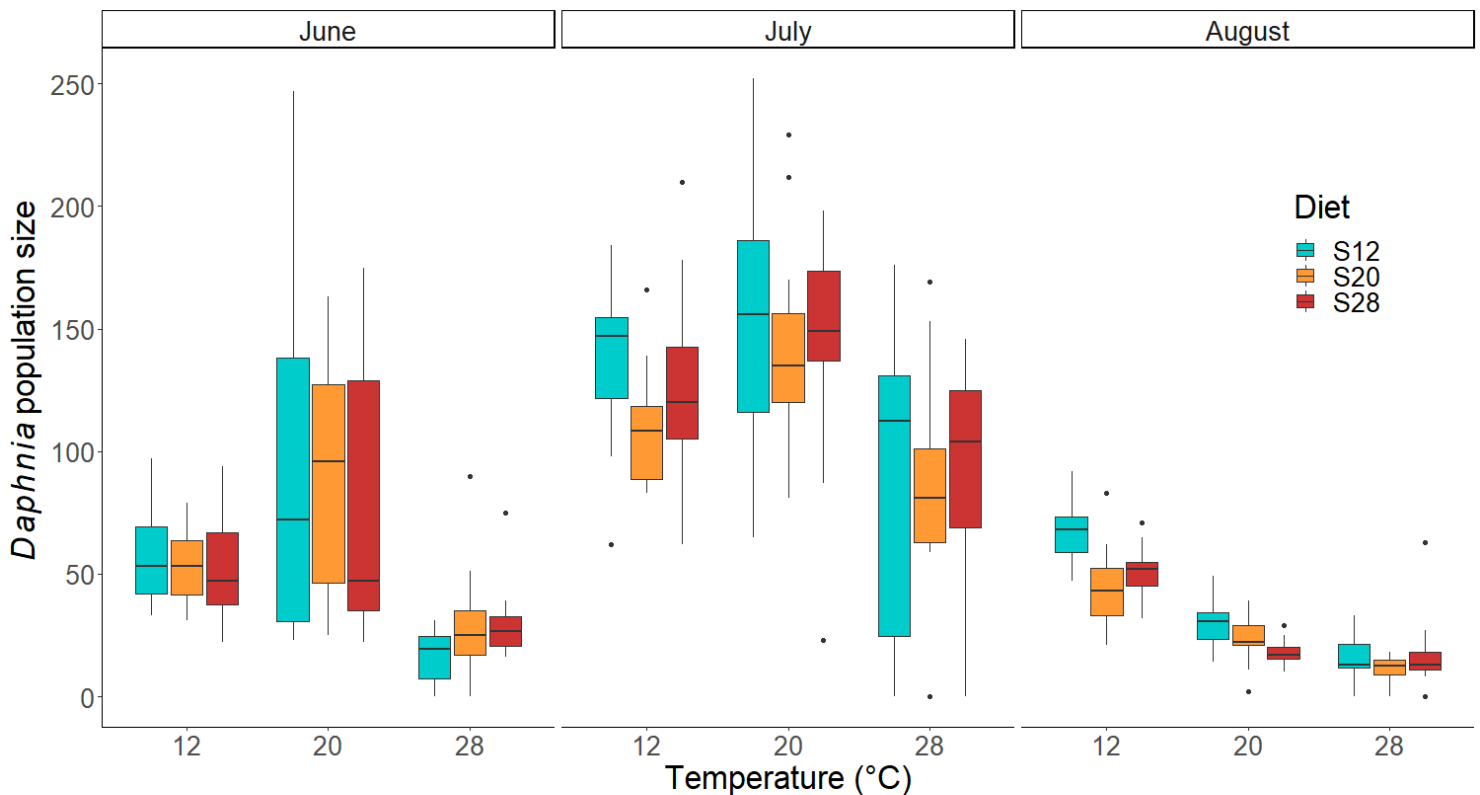
Figure A.4-1 The effects of rearing temperature (°C) and diet type on *D. pulex* population size. *D. pulex* were reared at 12, 20, and 28 °C (represented on the x-axis), and fed a diet of *S. obliquus* reared at 12 °C (S12), 20 °C (S20), or 28 °C (S28; represented by boxplot coloration).

	df	df_{Den}	Sum Sq.	Mean Sq.	F	p
				Test		

All months

	df	df_{Den}	Sum Sq.	Mean Sq.	F	p
Diet	2	400	3.98×10^3	1.99×10^3	1.926	1.47×10^{-1}
Rearing Temperature	2	3	2.90×10^4	1.46×10^4	14.074	**$2.43 \times 10^{-2*}$**
Month	2	400	6.00×10^5	3.00×10^5	290.255	**$2.20 \times 10^{-16***}$**
Diet:Temp	4	400	8.65×10^3	2.16×10^3	2.093	8.10×10^{-2}
Diet:Month	4	400	5.45×10^3	1.36×10^3	1.317	2.63×10^{-1}
Temp:Month	4	400	6.01×10^4	1.50×10^4	14.548	**$4.24 \times 10^{-11***}$**
Diet:Temp:Month	8	400	8.91×10^2	1.11×10^2	0.108	1.00×10^{-1}

June

	df	df_{Den}	Sum Sq.	Mean Sq.	F	p
Diet	2	134	6.79×10^2	3.39×10^2	0.256	7.74×10^{-1}
Rearing Temperature	2	134	8.75×10^4	4.37×10^4	33.012	**$2.21 \times 10^{-12***}$**
Diet:Temp	4	134	3.07×10^2	7.68×10^2	0.580	6.78×10^{-1}

July

	df	df_{Den}	Sum Sq.	Mean Sq.	F	p
Diet	2	132	4.87×10^3	2.43×10^3	1.574	2.11×10^{-1}
Rearing Temperature	2	3	1.08×10^4	5.41×10^3	3.500	1.65×10^{-1}
Diet:Temp	4	132	3.79×10^3	9.47×10^2	0.612	6.55×10^{-1}

August

	df	df_{Den}	Sum Sq.	Mean Sq.	F	p
Diet	2	131	2.78×10^3	1.39×10^3	12.564	**$1.02 \times 10^{-5***}$**
Rearing Temperature	2	3	2.19×10^4	1.10×10^4	99.137	**$1.22 \times 10^{-3**}$**
Diet:Temp	4	131	2.77×10^3	6.93×10^2	6.265	**$1.21 \times 10^{-4***}$**

*p<0.05, **p<0.01, ***p<0.001

A.5 The average quantity (ug) of fatty acids for *Chaoborus americanus* in each temperature treatment for types of saturation, fatty acids of interest, and omega-3 and omega-6 polyunsaturated fatty acids.

	Temp (°C)	Diet Type (°C)	Total Fat	Saturation	Fatty Acid	Omega	$\bar{x}$ (ug)	SD
Long-term experiment								
	12	12	Total				1.09×10^{-2}	4.29×10^{-3}
				MUFAs			2.84×10^{-3}	1.74×10^{-3}
				PUFAs			3.89×10^{-3}	2.09×10^{-3}
				SFAs			4.13×10^{-3}	4.61×10^{-4}
					ALA		1.21×10^{-3}	5.27×10^{-4}
					ARA		6.22×10^{-4}	3.89×10^{-4}
					DHA		4.34×10^{-5}	6.14×10^{-5}
					EPA		1.12×10^{-3}	6.51×10^{-4}
					LIN		8.08×10^{-4}	4.77×10^{-4}
					Oleic		1.70×10^{-3}	9.19×10^{-4}
						Omega-3	4.05×10^{-4}	6.16×10^{-4}
						Omega-6	1.79×10^{-4}	3.60×10^{-4}
						Omega-3: Omega-6	1.75	0.174
	12	20	Total				9.50×10^{-3}	7.88×10^{-4}
				MUFAs			2.38×10^{-3}	6.11×10^{-4}
				PUFAs			3.41×10^{-3}	5.46×10^{-4}
				SFAs			3.71×10^{-3}	3.69×10^{-4}
					ALA		1.04×10^{-3}	1.54×10^{-4}
					ARA		5.22×10^{-4}	1.44×10^{-4}
					DHA		0.00×10^{0}	0.00×10^{-0}
					EPA		1.00×10^{-3}	8.67×10^{-5}
					LIN		7.19×10^{-4}	1.86×10^{-4}
					Oleic		1.47×10^{-3}	3.31×10^{-4}

Temp (°C)	Diet Type (°C)	Total Fat	Saturation	Fatty Acid	Omega	$\bar{x}$ (ug)	SD
					Omega-3	3.44×10^{-4}	5.04×10^{-4}
					Omega-6	1.55×10^{-4}	2.89×10^{-4}
					Omega-3: Omega-6	1.70	0.234
Short-term experiment							
12	12	Total				3.29×10^{-2}	1.5×10^{-2}
			MUFAs			9.98×10^{-3}	5.10×10^{-3}
			PUFAs			1.24×10^{-2}	5.29×10^{-3}
			SFAs			1.05×10^{-2}	4.63×10^{-3}
				ALA		4.22×10^{-3}	1.88×10^{-3}
				ARA		1.98×10^{-3}	9.64×10^{-4}
				DHA		7.81×10^{-5}	6.89×10^{-5}
				EPA		3.58×10^{-3}	1.40×10^{-3}
				LIN		1.56×10^{-3}	6.64×10^{-4}
				Oleic		4.53×10^{-3}	2.25×10^{-3}
					Omega-3	1.37×10^{-3}	2.02×10^{-3}
					Omega-6	4.90×10^{-4}	8.41×10^{-4}
					Omega-3: Omega-6	2.13	0.333
12	20	Total				2.07×10^{-2}	9.30×10^{-3}
			MUFAs			5.45×10^{-3}	3.73×10^{-3}
			PUFAs			6.06×10^{-3}	3.26×10^{-3}
			SFAs			9.17×10^{-3}	2.30×10^{-3}
				ALA		2.01×10^{-3}	1.51×10^{-3}
				ARA		9.83×10^{-4}	4.40×10^{-4}
				DHA		3.70×10^{-5}	5.24×10^{-5}
				EPA		1.86×10^{-3}	7.38×10^{-4}
				LIN		7.45×10^{-4}	2.49×10^{-4}
				Oleic		2.61×10^{-3}	1.67×10^{-3}

Temp (°C)	Diet Type (°C)	Total Fat	Saturation	Fatty Acid	Omega	$\bar{x}$ (ug)	SD
					Omega-3	6.71×10^{-4}	1.07×10^{-3}
					Omega-6	2.22×10^{-4}	4.10×10^{-4}
					Omega-3: Omega-6	2.16	0.463
20	12	Total				3.14×10^{-2}	5.73×10^{-3}
			MUFAs			9.66×10^{-3}	1.80×10^{-3}
			PUFAs			1.06×10^{-2}	2.24×10^{-3}
			SFAs			1.12×10^{-2}	2.11×10^{-3}
				ALA		3.40×10^{-3}	1.11×10^{-3}
				ARA		1.80×10^{-3}	2.89×10^{-4}
				DHA		2.98×10^{-5}	5.96×10^{-5}
				EPA		2.97×10^{-3}	7.36×10^{-4}
				LIN		1.39×10^{-3}	2.60×10^{-4}
				Oleic		4.82×10^{-3}	1.04×10^{-3}
					Omega-3	1.13×10^{-3}	1.57×10^{-3}
					Omega-6	4.37×10^{-4}	7.01×10^{-4}
					Omega-3: Omega-6	1.93	0.485

A.6 Analysis of Variance (ANOVA) and linear mixed effects models for the short- and long-term experiments testing the effects of diet type and temperature on fatty acid profiles in *Chaoborus americanus*.

	df	df$_{Den}$	Sum Sq.	Test Mean Sq.	F	p

Effects of diet type

Long-term experiment
Total fat

	df	df$_{Den}$	Sum Sq.	Mean Sq.	F	p
Diet type	1		1.16×10^{-6}	1.86×10^{-6}	0.195	0.702
Residuals	2		1.90×10^{-5}	9.50×10^{-6}		

Saturation type

	df	df$_{Den}$	Sum Sq.	Mean Sq.	F	p
Saturation	2	4	3.83×10^{-6}	1.92×10^{-6}	3.705	**0.008**[**]
Diet type	1	2	1.01×10^{-7}	1.01×10^{-7}	0.195	0.862
Sat. x Diet	2	4	2.40×10^{-9}	1.18×10^{-9}	0.002	0.047

Fats of interest

	df	df$_{Den}$	Sum Sq.	Mean Sq.	F	p
Fatty acid	5	10	5.71×10^{-6}	1.14×10^{-6}	24.546	**2.58 x 10^{-5}**[***]
Diet type	1	2	5.10×10^{-9}	5.12×10^{-9}	0.110	0.772
Fat x Diet	5	10	2.11×10^{-8}	4.22×10^{-9}	0.091	0.992

Omega-3:Omega-6

	df	df$_{Den}$	Sum Sq.	Mean Sq.	F	p
Diet type	1		3.00×10^{-3}		0.071	0.815
Residuals	2		8.48×10^{-2}			

Short-term experiment
Total fat

	df	df$_{Den}$	Sum Sq.	Mean Sq.	F	p
Diet type	1		1.79×10^{-4}	1.79×10^{-4}	1.000	0.391
Residuals	3		5.38×10^{-4}	1.79×10^{-4}		

Saturation type

	df	df$_{Den}$	Sum Sq.	Mean Sq.	F	p
Saturation	2	6	1.16×10^{-5}	5.80×10^{-6}	21.589	**0.002**[**]
Diet type	1	3	2.69×10^{-7}	2.68×10^{-7}	0.999	0.391
Sat. x Diet	2	6	1.50×10^{-5}	7.49×10^{-6}	27.856	**9.19 x 10^{-4}**[***]

	df	df_{Den}	Sum Sq.	Mean Sq.	F	p
				Test		
Fats of interest						
Fatty acid	1	23	1.39×10^{-5}	1.39×10^{-5}	6.708	**0.016**[*]
Diet type	1	5	4.23×10^{-6}	4.23×10^{-6}	2.040	0.217
Fat x Diet	1	23	5.84×10^{-7}	5.84×10^{-7}	0.282	0.601
Omega-3:Omega-6						
Die type	1		1.55×10^{-3}	1.55×10^{-3}	0.012	0.924
Residuals	3		4.36×10^{-1}	1.45×10^{-1}		

Effects of temperature

Short-term experiment
Total fat

	df	df_{Den}	Sum Sq.	Mean Sq.	F	p
Rearing Temperature	1		3.65×10^{-6}	3.65×10^{-6}	0.033	0.863
Residuals	5		5.50×10^{-4}	1.10×10^{-4}		
Saturation type						
Saturation	2	10	9.67×10^{-6}	4.84×10^{-6}	8.083	**0.008**[**]
Rearing Temperature	1	5	1.99×10^{-8}	1.99×10^{-8}	0.033	0.862
Sat. x Temp.	2	10	5.05×10^{-6}	2.52×10^{-6}	4.216	**0.047**[*]
Fats of interest						
Fatty acid	1	33	3.78×10^{-5}	3.78×10^{-5}	16.890	**2.46 x 10**$^{-4}$[***]
Rearing Temperature	1	10	6.00×10^{-6}	6.00×10^{-9}	0.003	0.960
Fat x Temp.	1	33	6.04×10^{-7}	6.04×10^{-7}	0.270	0.607
Omega-3:Omega-6						
Rearing Temperature	1		6.63×10^{-2}	6.63×10^{-2}	0.358	0.576
Residuals	5		9.27×10^{-1}	1.85×10^{-1}		

*p<0.05, **p<0.01, ***p<0.001