THE THERMAL ECOLOGY OF POPULATIONS AND ECOSYSTEM SERVICES IN A
CHANGING WORLD

by

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Abstract

A central goal of ecology is to understand what drives the abundance, distribution and diversity of life on Earth. For centuries, biologists have been addressing these questions from a variety of perspectives, and yet we still lack a coherent and mechanistic understanding of what drives these patterns. One process that is shared by all of life on Earth is metabolism. The development and testing of metabolic scaling theory (MST), which formalizes relationships between body size, temperature and metabolism, has revealed remarkable generality in the way that organisms respond to the environment. In spite of extensive documentation of cross-species metabolic patterns, we still lack evidence for how metabolic constraints propagate from the fine to the broad organizational scales. The large gap in our understanding at the level of populations presents a critical challenge for MST. I have combined theory, experiments and data synthesis to test the metabolic underpinnings of biodiversity and its implications for human well-being. Results showed that the temperature-dependence of population dynamics can be predicted from the temperature-dependence of individual metabolism, thus lending strong support for the role of energetic constraints in governing population growth and abundance. Further, I showed that variation in ecologically important traits such as body size in aquatic species assemblages has important implications for a valuable ecosystem service: nutritional benefits from seafood. This approach has revealed that understanding what generates and maintains aquatic biodiversity has direct and immediate consequences for human well-being.
Lay Summary

Metabolic rate sets the pace of life. As temperatures increase, so do the metabolic rates of organisms. Focusing on the relationship between temperature, body size and metabolism has revealed remarkable generality in the way that organisms respond to the environment. I have tested and extended a general theory, metabolic scaling theory (MST), to address longstanding questions about the abundance and distribution of life on Earth. What are the relationships between individual performance and the non-living environment that determine the characteristics of individuals and populations? I found that we can use relationships between individual body size and metabolic rate to understand how quickly, and to what size populations grow. Further, I found that relationships between seafood species’ ecological characteristics, such as their body size, and their nutritional value can help us understand the connections between biodiversity and human well-being.
Preface

This thesis is based on original, independent work by the author (JRB).

A version of Chapter 2 has been accepted for publication in the *American Naturalist*. Bernhardt, J.R., Sunday, J.M., and M.I O’Connor. Metabolic theory and the temperature-size rule explain the temperature dependence of carrying capacity. *American Naturalist*, accepted. I (JRB) conceived and designed the study, collected, visualized, and analyzed the data and wrote the first draft of the manuscript. JMS and MIO contributed to writing the manuscript. MIO supervised the study. Undergraduate student Jane Yangel helped with algal counting and culturing.

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In Chapter 4, undergraduate student Kimmy Hofer helped with *Daphnia* counting and culturing.
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Dedication

For my sister, Nikki.

The amount of courage and compassion you have in a day is more than I can hope for in a lifetime.
Chapter 1: Introduction

1.1 Background

A central goal of ecology is to understand what drives the abundance, distribution and diversity of life on Earth. For centuries, biologists have been addressing these questions from a variety of perspectives, and yet we still lack a coherent and mechanistic explanation of what drives patterns of abundance and distribution. One process that is shared by all of life on Earth is metabolism. Every living thing must uptake, modify and allocate resources towards growth, maintenance and reproduction. The rate at which these processes occur is the metabolic rate. All ecological interactions involve exchanges of three currencies that fuel metabolism -- energy, matter and information (Brown et al. 2012). Energy is initially captured from sunlight and stored in chemical bonds used to power biological processes. Matter includes all the elements that make up biomass. Information is encoded in the DNA transmitted among generations, and the cues that organisms use to make sense of their environment. Metabolic scaling theory (MST) posits that temperature and body size impose universal constraints on the flux, storage and turnover of energy, matter and information (Brown et al. 2004, 2012). At its core, the premise is that principles of chemistry, physics and biology operating on metabolic processes at lower levels of biological organization (i.e. molecules and cells) constrain organismal performance and give rise to predictable patterns at higher levels (i.e. populations, communities and ecosystems). In this way, the focus on the shared metabolic processes of all life opens up a new possibility for a unified and predictive science of ecology. Metabolism may serve as a basis for a unified theory of ecology in the same way that genetics, as the study of the shared processes of replication,
translation and transcription of genetic material across all living things, has served as a foundation for a unified theory of evolution (Brown et al. 2004).

The development and testing of MST has revealed remarkable generality in the way that organisms respond to temperature (Enquist et al. 1998, Gillooly et al. 2001, Dillon et al. 2010). Syntheses across broad ranges of taxonomic diversity show that temperature has highly conserved and predictable effects on organismal metabolic rates (Gillooly et al. 2001, Chown and Nicolson 2004, Dillon et al. 2010). Patterns of size and temperature scaling (where ‘scaling’ refers to a relationship between two quantities, where a relative change in one quantity results in proportional relative change in the other, independent of the initial size of those quantities) are some of the most broadly observed patterns in nature (Huxley 1924, Kleiber 1932, Schmidt-Nielsen 1984, West et al. 2009). However, most of our understanding of how metabolic rate and other biological rates scale with temperature and body size comes from syntheses of organismal-level rates across species. While these patterns are clear and consistent with metabolic scaling theory, we still lack evidence for how the constraints propagate from the fine to the broad organizational scales. In particular, signals of these energetic constraints are less detectable at population and community levels (Fox and Morin 2001; Jiang and Morin 2004). This gap in our understanding of population-level effects of temperature presents a critical challenge for MST, and is key to the application of MST in explaining the abundance and distribution of populations and species.
1.2 Motivation

My thesis addresses two broad themes that integrate perspectives on energy, matter and information. The first theme (Linking individual performance with population persistence) focuses on energy and matter, while the second theme (Linking aquatic biodiversity, ecosystem services and human well-being) highlights the role of information associated with biodiversity.

Linking individual performance with population persistence

My thesis addresses a central challenge in ecology – to develop a mechanistic understanding of species abundance, distribution and diversity. Under the reality of a changing climate, never has this problem been more urgent. This remains a challenge because the diversity of biological responses to environmental variation at multiple scales makes predicting ecological outcomes a difficult task. One solution to this problem lies in harnessing our understanding of the predictable effects of temperature, energy and material fluxes in ecological processes. I explicitly integrate processes across levels of biological organization by empirically estimating how flows of energy and materials at the level of the individual cascade up to shape population growth rates, abundances and distributions of traits over gradients of temperature and resource supply.

Energetic constraints possibly operate at the population level (Savage et al. 2004). For a population to increase in abundance, the individuals in the population must capture more energy than they use for maintenance, and convert the excess into new biomass (growth) and individuals (reproduction). Since body size and temperature strongly influence organismal level processes, similar relationships might exist for population level parameters such as population growth rate, $r$, and carrying capacity, $K$, thus providing an energetic basis for demographic patterns in
populations (Savage et al. 2004). The evidence for population-level energetic constraints across temperature gradients is mixed (Huey and Berrigan 2001, Padfield et al. 2016, Luhring and DeLong 2017), and the relationship between temperature, organismal metabolism, demographic rates, and their outcomes remains unresolved. Understanding the effects of individual metabolism, and associated constraints on metabolic rates, on populations is a critical component of a unified theory of ecology. Population growth is related to mean fitness in an environment (Roughgarden 1979), and thus underlies a wide range of ecological and evolutionary processes.

Questions
Using a combination of theory, synthesis and experiments in planktonic mesocosms, I address several gaps in current MST. I focus on the links between processes operating at the individual and population levels because these links are the least well understood in the context of MST and yet critical to advancing a unified theory of ecology.

1) Do metabolic constraints on vital rates propagate directly from the individual level to the population level?

2) Do organisms’ physiological and demographic processes effectively average over their recent thermal history?

3) Is the temperature-size rule a consequence of increasing metabolic demands under warmer conditions?

4) Is temperature-driven variation in body size associated with variation in fitness?
Linking aquatic biodiversity, ecosystem services and human well-being

Obtaining sufficient and nutritious food is one of the greatest challenges facing humanity and understanding how aquatic ecosystems can help meet this goal is an urgent challenge for ecologists. The ecology of food security is not simply about predicting yields; it is about understanding the ecological conditions that lead to a stable supply of nutritionally diverse foods. I address this problem by identifying the ecological mechanisms associated with variability in micronutrient concentrations in edible fish tissues and the connections between aquatic biodiversity and the benefits to human well-being that it provides. Through data synthesis of key nutritional and ecological characteristics of edible aquatic species from all major ecoregions, I show that increasing aquatic biodiversity enhances human nutrition via portfolio effects, because aquatic species show distinct and complementary multi-nutrient profiles. By combining patterns of ecological variation and patterns in human consumption of species I explicitly link ecology to human well-being and explore the connections between aquatic biodiversity and human nutritional benefits.

Questions

While it has long been recognized that ecosystems provide essential benefits to humanity (Daily et al. 2009), a mechanistic understanding of the ecological processes underlying the supply of ecosystem services remains elusive. By drawing on biodiversity-ecosystem function theory (Loreau and Hector 2001), and quantitative metrics of human well-being, I address a key gap in our understanding of the relationship between ecosystem structure and ecosystem services.
5) What is the relationship between biodiversity in aquatic ecosystems and the benefits to human nutrition that these ecosystems provide?

6) Is variation in nutrient content among seafood species related to diversity in ecological traits and functions?

1.3 General approach

In this thesis, I have explored linkages between processes operating at multiple scales of biological organization, and the connections between these processes and human well-being. My approach combined theory, experiments and synthesis of global data. I examined the proximate and ultimate causes and consequences of temperature-dependent metabolic rate and body size -- the key parameters of MST. I tested critical predictions and assumptions of MST at the population level to identify the role of metabolic constraints in shaping population dynamics. I focused on manipulating temperature in aquatic mesocosms because its effect in natural systems is inescapable and biological responses to temperature are a key component of MST. As temperature increases, biological processes speed up – development happens faster; the rates of photosynthesis and respiration increase (Gillooly et al. 2002, Savage et al. 2004, Padfield et al. 2016). In this way, temperature can be considered a ‘master variable’ in ecological systems. Temperature in nature is variable, over time and space, so understanding the effects of temperature on individuals and populations is critical to explaining patterns of species’ abundance and distribution. In my experiments, I used aquatic mesocosms, in which I could easily control temperature and resource supply, thus enabling me to make robust and critical tests of theory.
In Chapter 2 I address a widely held, but untested, assumption that population abundance at carrying capacity declines with warming due to increased individual resource demands associated with higher metabolic rates. I experimentally test predictions of MST that relate population dynamics to individual metabolic rate and resource supply over a temperature gradient.

In Chapter 3 I test the prediction that population growth in thermally variable environments reflects the effects of non-linear averaging over the thermal performance curve (TPC) and show that the effects of temperature variation could be predicted by studying the biological mechanisms underpinning the curvature of the TPC.

In Chapter 4 I address the proximate and ultimate causes of variation in body size, a central parameter of MST. MST takes species’ body masses as a given, leaving unanswered the question of what drives patterns of body size in the first place. This lack of explanation of the ultimate cause of variation in key parameters such as body size or temperature dependence suggests that we need to expand the MST framework to include evolution. Here I address this problem in the context of the temperature-size rule (TSR). I ask whether temperature-dependent body size can be explained by a physiological compensating mechanism that balances resource supply with demand, and examine the fitness consequences of the phenotypically plastic TSR response.

Finally, in Chapter 5 I explore key linkages between aquatic ecosystems and human well-being in the context of seafood provisioning. I draw on biodiversity-ecosystem functioning theory to test the relationship between aquatic biodiversity and its multi-dimensional benefits to human
nutrition and well-being. I show that aquatic biodiversity enhances nutritional benefits, and that body size is strongly associated with nutritional quality, thus linking aspects of ecosystem structure and function with the benefits they provide.

This thesis advances our understanding of relationships between the abiotic environment and the characteristics of individuals, populations, and ecosystem services. By integrating measurements of metabolic fluxes at the individual level with plankton population dynamics, my approach unifies perspectives on energy flow with population ecology to create a more coherent and mechanistic science of global change. In addition, by connecting metrics of biodiversity (and the information associated with a variety of species and functional types) with metrics of human well-being, my work provides a mechanistic understanding of the processes underlying the benefits that ecosystems provide to people.
Chapter 2: Metabolic theory and the temperature-size rule explain the temperature dependence of population carry capacity

2.1 Summary
The temperature dependence of highly conserved subcellular metabolic systems affects ecological patterns and processes across scales, from organisms to ecosystems. Population density at carrying capacity plays an important role in evolutionary processes, biodiversity and ecosystem function, yet how it varies with temperature-dependent metabolism remains unclear. Though the exponential effect of temperature on intrinsic population growth rate, $r$, is well known, we still lack clear evidence that population density at carrying capacity, $K$, declines with increasing per-capita metabolic rate, as is often assumed in the metabolic theory of ecology (MTE). I experimentally tested whether temperature effects on photosynthesis propagate directly to population carrying capacity in a model species, the mobile phytoplankton *Tetraselmis tetrahele*. After maintaining populations at constant resource supply and temperatures (6 levels) for 43 days, I found that density declined with increasing temperature in a manner quantitatively consistent with predictions when models included temperature-dependent metabolic rates and temperature-associated body size shifts. My results demonstrate that warming reduces carrying capacity, and that temperature effects on body size and metabolic rate interact to determine how temperature affects population dynamics. These findings bolster efforts to relate metabolic temperature dependence to population and ecosystem patterns via MTE.

2.2 Introduction
At ecosystem scales, patterns of abundance, diversity and energy fluxes reflect the summed
performance of individual organisms. Organismal metabolic performance is sensitive to
temperature in a manner that explains substantial variation in ecosystem processes along
temperature gradients (López-Urrutia et al. 2006, Anderson-Teixeira et al. 2008, Yvon-Durocher
et al. 2012). In these examples from marine, freshwater and terrestrial systems, the temperature
dependence of highly conserved metabolic rates (e.g., photosynthesis and aerobic respiration)
emerges at community and ecosystem scales, even when temperature also affects population
dynamics and species interactions within these ecosystems. Understanding how temperature-
dependent metabolism modifies population dynamics and ultimately affects community and
ecosystem processes is a current challenge in an ecological science striving for unified
understanding across scales and levels of organization.

Density of a population at carrying capacity is a key concept linking population dynamics
to broader-scale patterns of biodiversity and population persistence (Damuth 1987, Savage et al.
abundance of a population under steady state conditions. Though it is a parameter in the simplest
logistic growth models, it is understood to be an emergent property that reflects density
dependent birth and death rates (Mallet 2012, Doebele et al. 2017, Uszko et al. 2017) or relative
growth and loss rates (McCann 2011, Gilbert et al 2014) that balance to maintain steady
population density through time. Density dependent birth and death rates limit population growth
rate at densities far below carrying capacity, at all but very low densities. As a theoretical
attribute of populations growing in an environment with limited resources, density at carrying
capacity is as important as intrinsic growth rate to understanding population dynamics (Gotelli
Efforts to scale up from how temperature affects individual performance to the outcomes of population, community and ecosystem processes, such as energy fluxes and food web dynamics, must assume a relationship between temperature and population carrying capacity (Savage et al. 2004, Allen et al. 2005, 2007, O’Connor et al. 2011, Gilbert et al. 2014, O’Gorman et al. 2017). The metabolic theory of ecology (MTE) postulates that the temperature dependence of widely shared metabolic rates (photosynthesis and respiration) drives temperature dependence of demographic rates (birth, death), leading to predictable effects of temperature on population growth (Savage et al. 2004, López-Urrutia et al. 2006, López-Urrutia 2008, Kremer et al. 2017) and community-level patterns (Savage et al. 2004, Allen et al. 2005, 2007, Meehan 2006, Osmond et al. 2017, Sentis et al. 2017). Savage et al. (2004) reasoned that carrying capacity would be expected to decline exponentially with increasing temperature proportionally to temperature-induced increases in per-capita metabolic resource demand, driven by increases in per-capita respiration rate. Subsequent models linking temperature-dependent metabolism to population and community level processes such as relative biomass across trophic levels have adopted this assumption (Allen et al. 2007, O’Connor et al. 2011, Gilbert et al. 2014, O’Gorman et al. 2017), while other studies have assumed no relationship between temperature and carrying capacity (Vasseur and McCann 2005, Sentis et al. 2017). These studies have shown how the assumed temperature dependence of carrying capacity of primary producers modulates food web responses to warming (O’Connor et al. 2011, Gilbert et al. 2014, Osmond et al. 2017, O’Gorman et al. 2017), although concomitant increases in nutrient supply with warming can buffer responses to temperature, by reducing resource limitation (O’Connor et al. 2011, Gilbert et al. 2014, O’Gorman et al. 2017). If the effect of temperature on carrying capacity is general, under known resource conditions, it could be extended to ecosystem level processes such as the
productivity of marine fisheries (Pauly and Christensen 1995, Sarker and Wiltshire 2017), geographic range expansion rates (Fronhofer and Altermatt 2015), or rates and direction of evolutionary change (Mallet 2012, Hendry 2017).

First-order MTE models and population dynamic models that relate per-capita metabolic rate to density at carrying capacity have assumed that body size and other traits that could influence carrying capacity do not covary with temperature (Savage et al. 2004, (O’Connor et al. 2011, Gilbert et al. 2014). However, this assumption contradicts substantial evidence that body size changes with temperature (Atkinson et al. 2003, Forster et al. 2012). Declines in body size of 1.7 – 3.3% °C⁻¹ (cell size in unicellular organisms) are widely observed responses to warming that can arise due to phenotypic plasticity or selection for smaller body sizes (Atkinson et al. 2003, DeLong 2012, Forster et al. 2012). This decline in body size is called the temperature size rule (TSR), and can mediate population dynamic responses to warming in the presence of consumers (Osmond et al. 2017). Warming-induced body size declines could alter predictions of population density over a temperature gradient if smaller individual sizes alleviate resource limitation and allow greater population densities than would be predicted for larger individuals.

I hypothesized that the temperature dependence of per-capita metabolic rates accurately predicts the decline in density at carrying capacity with increasing temperature within a population’s range of sub-optimal temperatures (Savage et al. 2004). To test this hypothesis, I maintained populations of the marine phytoplankton Tetraselmis tetrahele for 43 days over an experimental temperature gradient spanning 5°C – 38°C and estimated their per-capita oxygen consumption and production rates, mean cell size, intrinsic population growth rate (r) and steady state abundance (K). I expected cell size to decline with increasing temperature, and I tested the alternate hypothesis that declines in cell size would mitigate declines in density at carrying
capacity with increasing temperature. This is, to my knowledge, the first experimental test of hypotheses that explicitly contrast predictions based on the temperature dependence of metabolic rates and the TSR, under carefully controlled resource conditions that meet the assumptions of the logistic growth mode. I found that changes in metabolic rate and body size can together be used to quantitatively predict declines in carrying capacity with temperature according to the MTE, and I used these results to modify an existing general modeling framework (Savage et al. 2004) for how temperature dependent metabolism scales to population dynamics. My experiment provides novel empirical support for an often-made theoretical assumption in general population models about how temperature affects carrying capacity.

### 2.3 Methods

I modeled the effects of temperature on logistic population growth in terms of the temperature dependence of highly conserved metabolic rates (photosynthesis and aerobic respiration). I used the logistic model because it has been widely used to link the general body size- and temperature-dependence of metabolism to population and ecosystem patterns (Savage et al. 2004, Gilbert et al. 2014, O’Gorman et al. 2017). In the logistic growth model,

$$\frac{dN}{dt} = rN \left(1 - \frac{N}{K}\right)$$  \(\text{(1)}\)

\(N\) is the size of the population at time \(t\), \(r\) is the intrinsic growth rate and carrying capacity, \(K\), is the value of \(N > 0\) that makes \(dN/dt = 0\) (Verhulst 1838, Gotelli 1995).

Savage et al. (2004) applied the metabolic theory of ecology to relate logistic growth to the temperature dependence of metabolic rate by assuming that allocation of metabolic resources to new individuals (vs to somatic growth or maintenance) is independent of temperature.
Population level resource use is set by the total metabolic rate of the population, \( B_{\text{pop}} \), which is equal to the average individual metabolic rate, \( B_i \), summed over all individuals in the population. Carrying capacity occurs when the rate of resource use by the population equals the rate of resource supply to the population, \( P \), such that \( B_{\text{pop}} = P \). The number of individuals at carrying capacity, \( K \), is limited by average individual metabolic rate:

\[
K(M_i, T) \propto \frac{B_{\text{pop}}}{B_i(M_i, T)}
\]

where \( B(M_i, T) \) is the average individual metabolic rate at steady state, which varies with average individual body size \( (M_i) \) and temperature \( (T, \text{in Kelvin}) \). \( B_i \) scales with body mass and increases with temperature:

\[
B_i = b_{0i} M_i^{\frac{3}{4}} e^{-\frac{E_a}{kT}}
\]

where \( b_{0i} \) is a normalization constant, \( E_a \) is the activation energy of metabolism (West et al. 1997, Gillooly et al. 2001), \( k \) is Boltzmann’s constant and \( T \) is temperature in Kelvin. Equation 2 shows that an increase in individual metabolic rate will lead to a decrease in density, \( K(M, T) \), for a population at steady state \( (B_{\text{pop}} = P) \) with a constant, temperature-independent resource supply, \( P \). Combining Equations 2 and 3 shows how \( K(M,T) \) is predicted to scale directly with mass and temperature-dependent metabolic rate:

\[
K(M, T) \propto M_i^{\frac{-3}{4}} e^{\frac{-E_a}{kT}}.
\]

From here, I depart from previous models (Savage et al. 2004, O’Connor et al. 2011), and allow body size, \( M_i \), to decline linearly with increasing temperature (consistent with the TSR in ectotherms),

\[
M_i(T) = M_i(T_R)[1 - \beta(T - T_R)]
\]

(5)
where $\beta$ is the fraction by which body mass is reduced as temperature $T$ is increased by one degree, and $T_R$ is a reference temperature. This linear approximation of the TSR is appropriate for unicellular organisms (Atkinson et al. 2003, DeLong 2012, Forster et al. 2012). I can express my hypothesis that temperature-induced changes in size and temperature-dependent per-capita metabolic rate both determine how carrying capacity depends on temperature by substituting the temperature-dependent body size model (Equation 5) into the equation for the temperature dependence of $K$ (Equation 4):

$$K(M, T) \propto (M_i(T_R)[1 - \beta(T - T_R)])^{\frac{3}{4}} e^{\frac{E_a}{kT}}. \quad (6)$$

When mass is independent of temperature, ($\beta = 0$), the temperature dependence of $K$ is entirely captured by the temperature dependence of metabolism ($E_a$). However, decreases in body size with temperature, ($\beta > 0$), will reduce temperature-induced declines in $K$.

To linearize Equation 6 for comparison with experimental results, I first collapsed the temperature dependent mass term to $M_i(T)^{-3/4}$, re-arranged terms and then took the natural log of both sides:

$$\ln \left(K M_i(T)^{\frac{3}{4}}\right) \propto \frac{E_a}{kT}. \quad (7)$$

A critical test of the hypothesis that changes in carrying capacity can be predicted from the temperature dependence of metabolism and body size would be to test that the association between $\ln(KM_i(T)^{3/4})$ and temperature, in units of $1/kT$, yields a slope that is equal to $-E_a$. I tested this hypothesis experimentally with a photosynthetic autotroph, and I used $E_a = E_{GP}$, where $E_{GP}$ is the activation energy of gross photosynthesis (Brown et al. 2004, Gilbert et al. 2014, Padfield et al. 2016, O’Gorman et al. 2017). Gross photosynthesis (GP) is the total carbon fixed through photosynthesis, from which some portion is respired in the conversion of fixed
carbon to biomass (somatic growth or reproduction) or by-products. I used an Arrhenius function to describe the temperature dependence of metabolic rate (GP) for temperatures up to the estimated thermal optimum (25°C, Figure A1), and constrained our analysis to the increasing portion of the metabolic curve (Pawar et al. 2016). I focused on a range of sub-optimal temperatures because biological rates often decline sharply with increases in temperature beyond the thermal optimum. To model the full thermal response curve, a unimodal function such as the Sharpe-Schoolfield equation is often used (Schoolfield et al. 1981). Also, focusing on the relationship between temperature and population parameters over the range of suboptimal temperatures is appropriate for scaling from individual metabolic responses to temperature to broader community and ecosystem scales (Savage et al. 2004). When a population’s abundance reaches 0 due to thermal stress, another species with a different thermal niche is likely to be present and abundant, compensating for the functions performed by the population declining due to stress (Yvon-Durocher et al. 2012, Padfield et al 2017).

I used the temperature dependence of GP and body size in Equation 7 to predict how temperature dependent metabolic rate limits population growth rate and carrying capacity (Table 1). My purpose was to test this prediction empirically under controlled conditions for a single population with independent measures of oxygen metabolism and population dynamics. I tested the model assumption that energy allocation remains constant over a thermal gradient by estimating the temperature dependence of the ratio of gross photosynthesis to respiration (GP/R). I predicted that in the absence of systematic changes in body size with temperature, the temperature dependence of $K$ would be inversely proportional to the temperature dependence of GP, $E_{K,N} = -E_{GP}$ (here I use the subscript $K, N$ to refer to $K$ estimated in terms of number of individuals, to distinguish it from $K$ estimated in terms of biomass, which I will do later). If,
however, body size declines as predicted by the temperature size rule, then the observed decline in $K$ with temperature would be less than expected based on temperature dependent photosynthesis. In contrast, a plot of mass-normalized $\ln(KM_i(T)^{3/4})$ against $1/kT$ will yield a slope equal to $-E_{GP}$ if $K$ is estimated in terms of biomass, $K_{Bio}$. $K_{Bio}$ combines cell size and cell density, and so $\ln(K_{Bio})$ plotted against $1/kT$ should decline with a slope equal to $-E_{GP}$.

**Study system**

I tested my hypotheses in a closed phytoplankton microcosm system with fixed energy and nutrient supplies. I chose the flagellated marine chlorophyte, *Tetraselmis tetrahele* - a globally distributed coastal marine phytoplankton species. *T. tetrahele* is eurythermal, meaning populations are found at a broad range of temperatures below its thermal optimum around 25°C. It is an important photosynthetic food source in nearshore food webs with a generation time of $\sim$ 1 d at 20°C (Pena and Villegas 2005), making it a suitable species for microcosm studies and tests of metabolic scaling theory. The high mobility of this plankton species suggests that individuals can gain equal access to resources by moving throughout the microcosm, making it a good model species for meeting the assumptions underlying our hypotheses (Equation 7). I used a cultured strain obtained from the Canadian Centre for the Culture of Microorganisms (UW414), originally isolated off the coast of Vancouver Island, British Columbia, Canada. *T. tetrahele* were maintained in laboratory culture in ESAW medium (Enriched Seawater, Artificial Water, Harrison et al. 1980) in 30 mL glass test tubes at 16°C for one year on a 16:8 light:dark cycle under nutrient and light saturated conditions before the start of the experiments.

**Experimental conditions**
I set carrying capacity based on nitrate availability in experimental cultures. Though algal growth models have shown that nutrient- and light-limitation can have slightly different effects on equilibrium abundance and dynamics (Thomas et al. 2017, Uszko et al. 2017), I chose to limit one resource (nitrogen) to avoid potential complications associated with multiple limiting resources. I reduced the nitrate concentration by 55-fold relative to complete ESAW medium (final concentration 10μM) to ensure that nitrate concentration limited population densities. In pilot experiments, I confirmed nitrate limitation by comparing cell densities at steady state when grown at higher nitrate levels, and by observing no increase in abundance at higher light levels. To assess how population-level nitrate use at carrying capacity changes with temperature, I measured nitrate remaining in experimental microcosm water columns at steady state. At the end of the experiment, I filtered 2mL of experimental medium containing well-mixed phytoplankton onto GF/F filters. I assayed nitrate concentrations spectrophotometrically from the filtrate using a cadmium reduction method (Strickland and Parsons 1968; LaMotte Nitrate Nitrogen Test Kit) with a Turner Designs Trilogy fluorometer (Nitrate/Nitrite Module (P/N: 7200-074)).

**Sampling cell density and biomass**

I estimated biovolume (determined from area-by-diameter estimation) and cell density using a FlowCAM (FlowCAM VS Series, Fluid Imaging Technologies) at a flow rate of 0.3 ml/min. I converted biovolume to biomass (ug C = 0.109(biovolume)^0.991) (Montagnes et al. 1994) (Appendix A; Supplementary methods, Figures A5, A6).

**Estimating the temperature dependence of photosynthesis and respiration**
I determined the activation energy of photosynthesis and respiration over a temperature range from 8°C - 24°C by measuring oxygen production in the light and oxygen consumption in the dark using a 24-channel optical fluorescence oxygen system (Sensor Dish Reader SDR2, PreSens), equipped with a 24-chamber 200 uL glass microplate (Loligo Systems Aps, Tjele, Denmark). The oxygen sensor was placed in a temperature-controlled incubator (Panasonic M1R-154) with light at 80 umol/m²/s. Prior to measurements of net photosynthesis and respiration, 200 uL of well-mixed *T. tetrahele* cultures at densities of approximately 20 000 cells/mL, equivalent to carrying capacity at 16°C, were transferred from 30 mL test tubes to each microplate well. Wells were sealed with transparent PCR film (Thermo Scientific, Waltham, MA, USA), and measurements of oxygen concentrations were taken every 15 seconds over three hour periods, first in darkness and next in light, using the SDR v4.0 software (PreSens, Germany). Prior to oxygen flux measurements, sensor spots were calibrated with air-saturated water and water containing 2% sodium sulfite at each experimental temperature. After a four-hour gradual ramping from 16°C to each assay temperature, phytoplankton cells were acclimated for an hour in the dark prior to measurements.

I estimated gross photosynthesis (GP) as \( GP = NP + R \) at each temperature, where NP is net oxygen production rate (NP, mg O₂ mL⁻¹ hr⁻¹) and R is respiration rate, or net oxygen consumption rate (R, mg O₂ mL⁻¹ hr⁻¹). I measured rates on each plankton sample in dark and light conditions, and then estimated GP for each sample \((n = 18)\). I corrected for background microbial activity by subtracting from each respiration estimate the average oxygen flux from six control wells containing ESAW medium but no phytoplankton. I estimated per-capita mass-normalized metabolic rates \((B_i)\) by dividing oxygen fluxes by total population biomass estimated immediately before respirometry experiments. I used Ordinary Least Squares (OLS) regression
to estimate activation energies ($E$, Equation 3) from relationships between log transformed mass-normalized oxygen flux rates and temperature ($1/kT$).

*Estimating the temperature dependence of carrying capacity and cell size*

I initiated five replicate experimental populations of *T. tetrahele* in 30 mL glass test tubes containing 25 mL of 10μM nitrate ESAW medium at a density of 1000 cells/mL at 5°C, 8°C, 16°C, 25°C, 32°C, and 38°C. Experimental populations were held at constant temperature and light conditions (16:8h light:dark cycle; 60 umol/m²/s) until they reached steady state at all temperatures. I measured cell densities (cells/mL) and biovolumes (µm³/mL) from 250 µL samples every four days at the same time of day for 43 days until populations at all temperatures had reached carrying capacity (i.e. steady state).

To test my hypotheses (Equation 7), I estimated $K$ in terms of density ($K_N$, individual cells/mL, consistent with Equation 2) and in terms of population biomass ($K_{Bio}$). To estimate $K_N$, I fit a logistic growth model (Equation 1) to population abundance time series data using non-linear regression with the *nlsLM* function in R, which uses the Levenberg-Marquart optimization algorithm in the *minpack.LM* package (version 1.2.1, Elzhov et al. 2016). I used the *nls.multistart* package (version 1.0.0, Padfield and Matheson 2018) to iterate over 1000 different combinations of starting values drawn from a uniform distribution and chose the best model selected via the lowest AIC score. I quantified model fits graphically and by calculating Efron’s pseudo-$R^2$ values (noting that $R^2$ values are often a poor indicator of the performance of a non-linear model and require a different interpretation than for linear models; Spiess and Neumeyer 2010). I estimated uncertainty in model fits by bootstrap resampling the original time series data, refitting the logistic model 1000 times and plotting 95% confidence intervals (95% CI) (Figure A2). To
estimate $K_{Bio}$, I fit the logistic model to time series of population biomass. I estimated the fractional decrease in body size with each degree increase in temperature, $\beta$, with OLS regression, using average cell size in each population at carrying capacity and set $T_R = 5^\circ C$, the coldest temperature in my experiment. I used OLS regression to estimate the temperature dependence of $\ln(K_N/M_i(T)^{3/4})$ and $\ln(K_{Bio})$. I conducted all statistical analyses in R (version 3.4.1, R Core Team 2017), and used the purrr (version 0.2.3, Henry and Wickham 2017), broom (version 0.4.2, Robinson 2017) and modelr (version 0.1.1, Wickham 2017) packages to facilitate data manipulation and analysis.

2.4 Results

Temperature dependence of photosynthesis and respiration

The activation energies of mass-normalized oxygen fluxes in *T. tetrahele* were $E_{NP} = 0.24$ eV (95% CI: -0.02, 0.51, Table 1) for net photosynthesis, $E_R = 0.52$ eV (95% CI: 0.39, 0.66, Table 1) for respiration, and $E_{GP} = 0.33$ eV (95% CI: 0.20, 0.46, Table 1) for gross photosynthesis (Figure 1). The ratio of the two fluxes, $\ln(GP/R)$, decreased with increasing temperature with a temperature dependence of $E_{GP/R} = -0.20$ eV (95% CI: -0.32, -0.08, Table 1) (Figure A7).

Temperature dependence of cell size and carrying capacity

Population density and cell size at carrying capacity decreased with increasing temperature (Figures 2 and 3). Cell size decreased by 1.92% per degree increase in temperature (-1.57 ug C/°C, 95% CI -1.94, -1.21, Adjusted $R^2 = 0.81$, corresponding to a fractional decrease in body size, $\beta = 1.92%^{\circ}C^{-1}$, Table 1) (Figure 3A). This change was detected as early as day 4 in
the experiment and persisted until steady state (Figure A4).

Mass-normalized density at steady state, \(\ln(K_N M_i^{3/4})\), decreased with increasing temperature with a temperature dependence of \(E_{K,N} = -0.22\) eV (95% CI: -0.25, -0.18, Table 1) - less than my prediction from my first hypothesis, that \(E_{K,N} = -E_{GP} = -0.33\) eV (Equation 7, \(\beta = 0\), Figure 2A). When I used Equation 7 to predict density at steady state, \(\ln(K_N M_i(T)^{3/4})\), using \(\beta = 1.92^{\circ}C^{-1}\) and \(E_{GP} = 0.33\) eV (Figure 1), I predicted a density decline with increasing temperature \(E_{K,N} = -0.21\) eV (Figure 2A). This prediction is statistically indistinguishable from the empirical estimate of the temperature dependence of \(\ln(K_N M_i^{3/4})\) (Figure 2A). This result did not change when I used the observed average cell size in each population at carrying capacity in the mass normalization of \(K_N, K_N M_i(T)^{3/4}\), then the observed activation energy was \(E_{K,N} = -0.34\) eV (95% CI: -0.31, -0.37), which is statistically indistinguishable from the prediction that \(E_{K,N} = -E_{GP} = -0.33\) eV (Figure 2B).

I also estimated carrying capacity from time series of population biomass, which combines cell size and cell number. The \(\ln(K_{Bio})\) decreased with increasing temperature with a slope of \(E_{K,Bio} = -0.30\) eV (95% CI: -0.33, -0.26; Table 1, Figure 3B), which is statistically indistinguishable from the predicted slope of -0.33 eV. Population-level nitrate used at steady state, estimated as nitrate remaining in the microcosms, did not change with temperature (OLS regression slope = 0.055, 95% CI: -0.29, 0.19) (Figure 3C).

Including data from the warmer-than-optimal 32°C populations in the activation energy estimation for \(K_N\) and \(K_{Bio}\) introduced a non-linear decline in \(\ln(K)\) such that abundance declined much more rapidly above 25°C (Figure A3); therefore, these populations were not included in the linear fits (Figures 2, 3). I observed no population growth at 38°C and did not estimate \(K\) for these populations. These abundance declines are consistent with dominance of physiological
stress responses to temperature as it rises past the thermal optimum in *T. tetrahele*, which is approximately 25°C (Figure A1).

### 2.5 Discussion

Consistent with the metabolic theory of ecology that links general trends in individual metabolic rate to macroecological patterns in population and ecosystem patterns (Brown et al. 2004, Savage et al. 2004, Enquist et al. 2003), I found that carrying capacity varies with the temperature dependence of photosynthesis and the temperature dependence of body size in a model species, the chlorophyte *Tetraselmis tetrahele*. While \( K \) declined with warming, the concomitant reduction in body size of approximately \( \beta = 1.92\%\,^\circ\text{C}^{-1} \) meant that \( K \) did not decline by nearly as much as would have been predicted by MST when assuming a temperature-invariant body size. My results provide empirical support for the often-assumed negative relationship between carrying capacity and temperature mediated by increased per-capita metabolic rates (O’Connor et al. 2011, Rall et al. 2012, Gilbert et al. 2014), but also demonstrate that when linking metabolic rates to population dynamics, effects of temperature on body size cannot be ignored.

I empirically estimated an activation energy of \( E_{GP} = 0.33 \text{ eV} \) for gross photosynthesis, consistent with previously published estimates of the activation energy of photosynthesis in phytoplankton (López-Urrutia et al. 2006, Regaudie-de-Gioux and Duarte 2012, Yvon-Durocher and Allen 2012, Padfield et al. 2016). Recent studies have found that the temperature dependence of gross photosynthesis in phytoplankton constrains outcomes of population level ecological and evolutionary processes (Padfield et al. 2016, Schaum et al. 2017). In my study, this temperature dependence predicted the general decline in total biomass with warming, but
quantitatively, my observed population densities at warmer temperatures were higher than expected based on the temperature dependence of photosynthesis alone. However, when I considered the phenotypic decline in body size at warmer temperatures, and the expectation that smaller body sizes could allow greater density per unit population biomass, I was able to accurately predict density declines at higher temperatures. This suggests that a phenotypic change in body size, consistent with the widely observed temperature size rule, was associated with lower per-capita resource use that may have mitigated the effect of increased metabolic rates on density, but not total biomass. My finding that using a linear approximation of the temperature size rule (captured by $\beta$) accurately predicts declines in abundance with temperature suggests that in the absence of estimates of body size for all individuals in the population, a general relationship between size and temperature for a system may provide a reasonable estimate of the indirect effects of temperature-dependent body size on carrying capacity. Other undetected phenotypic changes may have occurred during the course of the experiment, which could also change the relationship between instantaneous metabolic demand and carrying capacity. Nevertheless, the link between temperature, metabolic rate and carrying capacity I present here provides a mechanistic lens for understanding previous studies that have reported similar negative temperature dependence of carrying capacity and assumed, rather than measured, metabolic rates (Alto and Juliano 2001, West and Post 2016).

Divergent responses of population density and population biomass to temperature have implications for higher-order processes in ecosystems. While total biomass is often considered an important estimate of ecosystem functions, the number and size of individuals influences demographic processes and their outcomes. Theoretical analyses that have extended the metabolic theory of ecology to population dynamics in consumer-resource systems have shown
that the temperature-size rule can stabilize consumer-resource systems over temperature gradients (Osmond et al. 2017). The effects of smaller body size on population growth rate and consumer-resource interactions counteract the expected effects of temperature on growth rates and carrying capacity, thereby allowing greater persistence of systems in warming environments compared to systems in which body size does not change with temperature (Osmond et al. 2017, Uszko et al. 2017, Sentis et al. 2017). The dynamical consequences of body size shifts are an important link between organismal responses to temperature and ecosystem level patterns of diversity and energy flux. This stabilizing effect of the temperature-size rule on dynamics suggests that as per-capita metabolic rates increase with warming, population collapses that might be expected due to destabilizing dynamics do not occur.

Relating my findings to other reports from laboratory and field assessments of population abundance as a function of temperature is not straightforward without clear evidence that observations met the conditions assumed by Equation 7, namely that resource supply remained constant across temperature treatments, the temperature dependence of the metabolic rate most relevant to limiting growth is known, and body size did not change with temperature. Some studies in microbial systems have reported weak or no response of carrying capacity to temperature (Zwietering et al. 1991, Urit et al. 2013, Arandia-Gorostidi et al. 2017). The ability of bacteria in anaerobic systems to maintain density over a thermal gradient may reflect their use of multiple metabolic pathways, each with a distinct temperature dependence – not the highly conserved photosynthesis and aerobic respiration used by most phytoplankton. Further, these studies report only cell density, and do not provide observations on metabolic rate or cell size over the thermal gradient, so they do not provide data sufficient to test hypotheses derived from Equation 7. Other studies report increasing, decreasing, or unimodal changes in abundance.
correlated with temperature under conditions in which resource supply was not controlled (Isaac et al. 2011, O’Gorman et al. 2017, Meehan 2006) or controlled but unknown (Fox and Morin 2001, Jiang and Morin 2004).


Consistent with the predictions of MTE (Savage et al. 2004), my findings suggest that per-capita resource demand increased in warm conditions, and nutrient use efficiency increased at larger cell sizes and colder conditions. Given the same supply of resources ($P$) to all populations across my experimental temperature gradient, increasing temperatures resulted in lower population abundances, in which each individual was fluxing energy and materials at higher rates. I observed that population-level resource use (measured as nitrate remaining in the microcosms at steady state) was the same across all temperatures, despite higher population abundances and larger cell sizes in the cold, suggesting that warm populations had higher per-capita resource demands. This suggests that under cold conditions, *T. Tetrahele* is more efficient at converting the limiting nutrient into biomass (Marañón et al. 2013, 2018). This greater efficiency is also consistent with the temperature dependence of the ratio of GP/R (Figure A7), which suggests that at colder temperatures a smaller fraction of carbon fixed is respired immediately. Models of temperature-dependent resource allocation support this finding: as temperatures increase, phytoplankton are predicted to decrease investment in phosphorus-rich ribosomes and increase investment in nitrogen-rich light-harvesting machinery, leading to higher per-capita nitrogen demand (Toseland et al. 2013). In addition to the direct effects of temperature on per-capita
resource use, temperature may also affect per-capita resource use indirectly via shifts in cell size. In marine phytoplankton, maximum nutrient uptake rates increase isometrically with cell size (Marañón et al. 2013), while minimum nitrogen requirements scale with negative allometry (i.e. scale with a slope of ~0.87), meaning that larger cells are more mass-efficient at converting nutrients to biomass (Marañón et al. 2013).

Here I showed that carrying capacity of photosynthetic autotrophs declined with increasing temperature as simple functions of the increase in per-capita gross photosynthetic rate and temperature-driven decline in body size. I extended metabolic theory models to include predictions that account for concomitant changes in body size with temperature described by the temperature-size rule. This work bolsters a key assumption in the metabolic scaling framework for how the temperature dependence from subcellular processes influences ecosystem processes, via population dynamics. My empirical system was intentionally simple and designed to meet the assumptions of a simple population model. The link between per-capita metabolic rates and demographic outcomes will likely be more complex when multiple species or longer temporal dynamics are examined. For example, changes in allocation of resources to reproduction, varying resource dynamics, or evolution may alter the relationship between instantaneous metabolic rates and demographic processes (Michaletz et al. 2014, Padfield et al. 2016, Osmond et al. 2017, Uszko et al. 2017, Kirk et al. 2018). As the metabolic theory of ecology continues to expand and incorporate additional complexity and ecological processes, such complexities can be modeled and understood in relation to the simpler dynamics I have demonstrated here. In this way, the metabolic theory of ecology serves as a framework to unite dynamical models and empirical evidence for ecological responses to temperature, from organisms to ecosystems.
2.6 Figures

Figure 2.1 Mass-normalized metabolic rates of *T. tetrahele* increase with temperature.

Mass-normalized metabolic rates \(B_i M_i^{-3/4}\) of *T. tetrahele* increased with temperature for gross photosynthesis (A) and for respiration (B). Points are shown at medium opacity to indicate overlap, shaded bands refer to 95% confidence intervals from OLS regression. For ease of interpretation, a secondary horizontal axis (upper axis) shows temperature in °C.
Figure 2.2 Carrying capacity decreased with temperature.

A) Carrying capacity (cells mL\(^{-1}\) ug C), \((K_NM_{i}^{3/4})\), decreased with increased temperature. OLS regression of observed density over the temperature gradient (solid line) (shaded area representing 95% confidence intervals) diverged from the model prediction (dotted line) based on Equation 7 assuming temperature-independent body mass \((\beta = 0)\). The model prediction based on Equation 7 that included the observed decline in body size with increasing temperature \((\beta = 1.92\% \degree C^{-1})\), thick dashed line) matched OLS regression to observed results. B) Mass normalized carrying capacity (cells mL\(^{-1}\) ug C), \((K_{N}M_{i}(T)^{3/4})\), decreases with temperature. In panel B, \(M_{i}\) varies with temperature and corresponds to the average cell biomass in each replicate population at carrying capacity. For ease of interpretation, a secondary horizontal axis (upper axis) shows temperature in \(^\circ C\).
Figure 2.3 Cell size, estimated population biomass and nitrate remaining in microcosms at carrying capacity.

Cell size (ug C cell⁻¹) (A), estimated population biomass ln(K_{Bio}) (ug C mL⁻¹) (B) and nitrate remaining in microcosms at carrying capacity across a temperature gradient from 5°C to 25°C (shaded bands in all panels represent 95% confidence intervals from OLS regression). In panel A, observed cell sizes are shown (large circles represent population mean cell size, n = 5 populations, small grey dots represent individual cell size estimates, subsample of n = 30 per population, pooled across populations to show the spread in cell size, OLS regression fit to population-level data). For ease of interpretation, a secondary horizontal axis (upper axis) shows temperature in °C.
Chapter 3: Non-linear averaging of thermal experience predicts population growth rates in a thermally variable environment

3.1 Summary

As thermal regimes change worldwide, projections of future population and species persistence often require estimates of how population growth rates depend on temperature. These projections rarely account for how temporal variation in temperature can systematically modify growth rates relative to projections based on constant temperatures. Here, I tested the hypothesis that population growth rates in fluctuating thermal environments differ from growth rates in constant conditions, and that the differing thermal performance curves (TPCs) can be predicted quantitatively. With experimental populations of the green alga *Tetraselmis tetrahele*, I show that nonlinear averaging techniques accurately predicted increased as well as decreased population growth rates in fluctuating thermal regimes relative to constant thermal regimes. I extrapolate from these results to project critical temperatures for population growth and persistence of 89 phytoplankton species in naturally variable thermal environments. These results advance our ability to predict population dynamics in the context of global change.
3.2 Introduction

Organisms live in variable environments. Demographic rates and outcomes that integrate
temporal or spatial environmental variation may differ substantially from what might be
predicted based on short-term physiological responses to constant, non-varying experimental
environments. For example, population growth rates are predicted to vary with temperature as
described by their thermal performance curve (TPC), often equated to an organisms’ thermal
niche (Figure 1A). Parameters of the thermal niche, such as the upper and lower critical
temperatures for population growth, or the optimal temperatures for maximum rates of
population growth, are important parameters in the large and growing body of synthesis research
that links physiological processes with projected population responses to climate change (Sunday
et al. 2012, Vasseur et al. 2014, Buckley et al. 2014). However, elements of the thermal niche are
often documented in physiological assays that use constant laboratory environments. Thermally
variable environments can lead to population growth rates over time that differ substantially
from estimates based on the average temperature over the same time period (Gonzalez and Holt
2002, Lawson et al. 2015). This difference complicates projections of population performance
based on physiological assays under constant conditions (Estay et al. 2014), prompting calls for
ecologists to explicitly incorporate environmental variation into predictions and models of
population and species’ performance in the field (Dowd et al. 2015). Because temporal patterns
of environmental variability differ across regions and the lifespans of organisms, an approach
that allows quantitative scaling from thermal performance curves of population growth generated
under constant laboratory conditions to population performance in a variable thermal regime may
be particularly useful for understanding patterns of abundance and distribution, and species’ responses to climate change.

Biological responses to environmental variation depend on whether the relationship between performance and an environmental gradient is linear or nonlinear (Ruel and Ayres 1999, Drake 2005, Lawson et al. 2015), and if nonlinear, whether it is accelerating with increasing temperature, or decelerating (Figure 1A). When performance, $P$, changes nonlinearly with environmental conditions, $E$, time-averaged performance in a variable environment $\bar{P}(E)$ does not necessarily equal performance at the mean environmental condition $P(\bar{E})$. This fact, captured by the well-known mathematical rule ‘Jensen’s inequality’, leads to clear predictions about how environmental variability should affect performance over time (Jensen 1906, Ruel and Ayres 1999). Jensen’s inequality states that if $P$ is a nonlinear function of $E$, then $\bar{P}(E) > P(\bar{E})$ where $P(\bar{E})$ is accelerating (i.e. positive second derivative) and $\bar{P}(E) < P(\bar{E})$ where $P(\bar{E})$ is decelerating (i.e. negative second derivative) (Figure 1B). In the context of temperature, the relationship between organismal or population performance and temperature, captured in the thermal performance curve (Figure 1A), is almost always nonlinear (Schulte et al. 2011) so the often implicitly assumed linear relationship between environment and population growth in demographic models is inadequate to describe population dynamics over temperature gradients (Lande et al. 2003). The potential ecological and evolutionary effects of Jensen’s inequality have been shown in several recent studies (Martin and Huey 2008, Vasseur et al. 2014, Estay et al. 2014, Denny 2017). Yet, ecologists struggle to incorporate thermal variability when making predictions about the effects of temperature on growth, abundance, and distributions of species in
nature, often assuming that species’ thermal experiences are well represented by the mean temperature of their environment.

The typical shapes of TPCs (Figure 1A) (Dell et al. 2011), with an accelerating phase at lower temperatures and a decelerating phase at higher temperatures within a thermal performance curve suggests positive effects of thermal variation at low temperatures and negative effects at high temperatures (Dowd et al. 2015, Denny 2017). Current estimates of the consequences of temporal thermal variability for population-level performance such as population growth rate have assumed a certain shape to the curve (i.e. a Gaussian rise and a parabolic fall; (Vasseur et al. 2014)) thus forcing certain outcomes of temporal variability. New evidence suggests that the shape and skew of the TPC can vary substantially among species, phenotypes, or contexts (Thomas et al. 2012), leading to potentially more nuanced responses to environmental variation that may be predicted from empirical thermal performance curves. To date, empirical tests of how temporal temperature variability affects population growth rates have been based on tests at only two temperatures (Bozinovic et al. 2011). This restricted sampling of the TPC has precluded testing of quantitative predictions based on the curvature of the TPC.

Here I tested whether population growth in a temporally variable thermal environment reflects the effects of nonlinear averaging of performance at each temperature experienced. For a fast-growing green alga, I tested whether TPCs for population growth generated under constant conditions could predict the outcome of population growth in thermally fluctuating environments. I hypothesized that for populations of this alga, which has overlapping generations and short generation times, population growth measured over several generations would reflect
the instantaneous effects of time-averaging of acute thermal responses. Alternatively, if time-dependent stress or acclimation effects that depend on recent thermal history modify growth rates in fluctuating environments (Niehaus et al. 2012, Kingsolver et al. 2015, Kremer et al. 2018), then population performance in naturally variable environments may not be predicted directly from TPCs generated under constant laboratory conditions, and would require a more detailed understanding of the mechanisms and time-course of thermal niche plasticity.

Following from Jensen’s inequality, I predicted that increased temperature variability over the range of cold temperatures to the left of the inflection point of the TPC (i.e. in the accelerating portion of the curve, where the second derivative of the TPC is positive; Figure 1B) would lead to higher growth rates relative to constant conditions, and that temperature variability in the range of warm temperatures to the right of the inflection point would be detrimental (i.e. where the second derivative of the TPC is negative; Figures 1B and 2A, dashed curve). Then, drawing on a global dataset of empirical TPCs for phytoplankton population growth rates, which vary in shape (skew and position) as well as geographic origin, I use nonlinear averaging (Equation 1) to estimate in situ population growth rates, given levels of in situ environmental variation at each species’ isolation location. I estimate the extent to which predicted growth rates at each isolation location differ when they are predicted using nonlinear averaging of fluctuating temperatures over time, as compared to when they are predicted based on mean annual temperatures only. By including a range of phytoplankton TPC shapes from a global distribution, I explore the consequences of considering thermal variability in projections of population growth rates, with implications for patterns of abundance and distribution.
3.3 Methods

Using nonlinear averaging to predict population growth in variable environments

When time series of temperatures experienced by organisms are available and population growth rate, \( r \), is given by a thermal performance curve, \( r = f(T) \), then the expected growth rate, \( E(r) \) averaged over time, \( t \), can be calculated by taking the average of the performance at individual time steps:

\[
E(r) = \frac{1}{t} \sum_{t=1}^{t} f(T_t)
\]  

(1)

where \( t \) indexes time.

Empirical records of environmental or body temperatures across time required to use Equation 1 are often not available; however, mean and variance of the distribution of environmental temperatures over a period of time may be more readily accessible. An alternative approach to estimating expected performance under variable conditions when only the mean and variance of the temperature distribution are available is to approximate expected performance under variable conditions using a Taylor approximation of the TPC (Equation S5, an approach that has been incorporated into scale transition theory (Chesson et al. 2005, Dowd et al. 2015, Denny 2017)). I explore expectations and results using this approach from scale transition theory as well, and compare results using both approaches, to increase the toolkit for ecologists with different kinds of temperature data available (see Appendix B).

Experimental quantification of TPCs in constant and fluctuating environments
I experimentally quantified acute TPCs in constant and varying thermal environments for *Tetraselmis tetrahele*, a globally distributed coastal marine phytoplankton species. I used acute TPCs estimated directly from experimental populations, rather than longer-term acclimatized TPCs, to match the time scale of temperature exposure under constant and fluctuating conditions and to simulate the effects of temperature variability in real time. The cultured strain used here was obtained from the Canadian Centre for the Culture of Microorganisms (UW414), and was originally isolated off the coast of Vancouver Island, British Columbia, Canada. The *T. tetrahele* was maintained in laboratory culture in ESAW medium (Enriched Seawater, Artificial Water, (Harrison et al. 1980)) at 16°C on a 16:8 light:dark cycle under nutrient and light saturated conditions before the start of the experiments.

I initiated twenty replicate experimental populations of *T. tetrahele* in 30 mL glass test tubes containing 20 mL of 10uM nitrate ESAW medium at a density of ~ 700 cells/mL under constant temperature conditions at 0°C, 5°C, 10°C, 15°C, 20°C, 24°C, 27°C, 29°C, and 32°C, and under fluctuating temperature conditions with the same mean temperatures as the constant conditions, but fluctuating ± 5°C; i.e. 0°C – 10°C, 5°C – 15°C, 10°C – 20°C, 15°C – 25°C, 19°C – 29°C and 22°C – 32°C. I created fluctuating temperature treatments by programming temperature-controlled incubators (Panasonic MR 154) to switch between the low and high temperature once per day (i.e. approximately 11.5 hours at the high temperature and approximately 11.5 hours at the low temperature, with 30 minutes of transition time in between each temperature cycle). I verified the temperature fluctuations in the experimental populations by measuring water temperatures inside test tubes with 20 mL of water at 1 minute intervals with iButton temperature loggers (Maxim/Dallas Semiconductor)). The fluctuation period of 11.5 hours
corresponds to approximately half a generation time at 20°C. To avoid experimental artifacts associated with confounding the temperature cycles with daily light cycles, we grew all experimental populations under 24-hour continuous light, at saturating light intensities of 150 umol/m²/s (see Figure B1). The source population was acclimated to 24-hour continuous light for four months prior to the experiment. I sampled each of the twenty replicate populations, with four replicates sampled destructively at each of five time points over the period corresponding to the exponential growth phase at each temperature. The intervals between sampling periods depended on the temperature, such that for the warmer temperatures (>15°C) sampling time points were condensed (up to 3 samples per day) to capture exponential growth, while at the colder temperatures (=<15°C) sampling intervals were spread out over longer time periods (i.e. one to five days between sampling points at 0°C and 5°C). Population abundances and cell biovolumes (using area-by-diameter estimation, ABD) were measured from 250 uL samples using a FlowCAM (flow rate = 0.3 ml/min; FlowCAM VS Series, Fluid Imaging Technologies).

Estimating the temperature dependence of population growth in constant and variable conditions

I estimated the temperature-dependence of population growth directly from the observed time series of population abundance over the temperature gradient (Palamara et al. 2014). I modeled the temperature-dependent intrinsic rate of population growth, \( r \), during the exponential growth phase as:

\[
N(t) = N(0)e^{r(T)t}
\]  

(2)

where \( N(t) \) is the number of individuals at time \( t \), and \( r(T) \) is given by Thomas et al. 2012:
\[ r(T) = ae^{bT} \left[ 1 - \left( \frac{T - z}{w} \right)^2 \right] \]  

(3)

using non-linear least squares regression with the \textit{nls.LM} function in the \textit{minpack.LM} package in R (Elzhov et al. 2016). Population growth rate, \( r \), is a function of temperature, \( T \), \( a \) and \( b \) are parameters from the Eppley curve (Eppley 1972) that together describe the increase in maximum observed population growth rates with temperature, \( z \) determines the location of the maximum of the quadratic portion of the function and \( w \) is the range over which the growth rate is positive (i.e. the thermal breadth). I also estimated the temperature dependence of population growth via an ‘indirect’ approach (Palamara et al. 2014) (Appendix B), in which we first estimated growth rates at each temperature separately, and then fit the TPC (Equation 3) to the growth estimates at each temperature. I present the results from this ‘indirect’ approach in the ESM (Figures S2 and S6). To test my hypothesis that performance at constant temperatures predicts performance in fluctuating thermal regimes, I then fit Equations 2 and 3 to the time series of population abundance in the variable experimental treatments, using \( T = \bar{T} \), the mean temperature in each treatment in the variable thermal regimes.

Importantly, this generalized TPC equation (Equation 3) does not force the TPC to take on any particular values of \( a \), \( b \) or \( z \) or \( w \), such that estimated TPCs can be either fully decelerating throughout the entire range of temperatures over which growth is positive, or can have any combination of accelerating and decelerating portions. I favored this TPC equation over others because of this flexibility, because it is parameterized with biologically meaningful parameters, and because has been used previously in studies of phytoplankton TPCs (Thomas et al. 2012,
For comparison, I fitted several other functional forms to the experimental dataset (e.g. a Gaussian x Gompertz function, as in (Martin and Huey 2008)) but did not find any better fits when we compared models via AIC.

I quantified four critical temperatures of the TPC (Equation 3) that define the thermal niche (Figure 1A): the optimal temperature for population growth, $T_{opt}$, the minimum temperature for positive population growth, $T_{min}$, the maximum temperature for positive population growth, $T_{max}$, and thermal niche breadth under constant conditions, $w$. Here I use $T_{min}$ and $T_{max}$ to denote the lower and upper limits of the thermal niche for population growth, and note that they are analogous to, but distinct from CTmin and CTmax which are the critical lower and upper limits for organism function (Lutterschmidt and Hutchison 1997). Since $T_{opt}$, $T_{min}$ and $T_{max}$ are not parameters of Equation 3, but rather features of the curve, I identified $T_{opt}$ via numerical optimization using the `optim` function in R and $T_{min}$ and $T_{max}$ by finding the roots of the TPC using the `uniroot` function in R. I quantified the analogs of these critical temperatures under thermally variable conditions and refer to them as the minimum mean and maximum mean temperatures for positive population growth under fluctuating conditions, $T_{\text{min}}^{\text{fluc}}$ and $T_{\text{max}}^{\text{fluc}}$, respectively, the mean temperature for optimal growth under fluctuating conditions, $T_{\text{opt}}$, and thermal niche breadth, $\bar{w}$. Because $T_{\text{min}}$ from the estimated curve could be below the freezing point of seawater -1.8°C, I used an additional metric of thermal breadth, in which we set $T_{\text{min}}$ to be -1.8°C if it was estimated to be below -1.8°C, because I assumed that *Tetraselmis tetrahele* cannot maintain positive population growth below the freezing point of seawater. I then
calculated the range of temperatures over which population growth rate is positive as the difference between $T_{\text{min}}$ and $T_{\text{max}}$.

To generate estimates of uncertainty in critical temperatures of the TPC (e.g. $T_{\text{opt}}$) under constant and variable conditions, we determined confidence intervals around fitted thermal performance curves using non-parametric bootstrapping of mean-centered residuals using the *nlsBoot* function with 999 iterations in the *nlstools* package in R. I calculated 95% confidence intervals as the range between the 2.5th and 97.5th quantiles.

To test my hypothesis that the performance in varying conditions can be explained by nonlinear averaging performance at each temperature experienced, my generated an expected TPC for *T. tetrahele* under thermally fluctuating conditions. I evaluated Equation 1 with $f(T)$ equal to the TPC fitted using Equation 3, for all values of $T$ between 0°C and 33°C (i.e. the entire TPC). I generated confidence intervals around the expected TPC under variable conditions by evaluating Equation 1 for each of the 999 bootstrapped constant-environment curves and calculating 95% confidence intervals as the range between the 2.5th and 97.5th quantiles (Figure 2A, dashed band).

Following Jensen’s inequality, I expected that increased temperature variability should increase population growth in the accelerating phase of the TPC and decrease population growth rate in the decelerating phase of the TPC (Figure 1), and that the shift between positive and negative effects of temperature variability should occur at the inflection point of the constant-temperature TPC. I predicted that increased range of variability should shift the lower and upper limits of the TPC under variable conditions ($\bar{T}_{\text{min}}$ and $\bar{T}_{\text{max}}$) to lower temperatures than $T_{\text{min}}$ and $T_{\text{max}}$ since
the TPC is left skewed. I expected the time-averaged maximum growth rate, $r_{max}$, to decrease under variable temperature conditions relative to constant conditions because $T_{opt}$ is always in a decelerating portion of the constant TPC curve. Finally, I expected the thermal breadth under fluctuating conditions, $\varpi$, to also decrease under fluctuating conditions if $T_{min}$ is close to freezing, thus preventing $T_{min}$ from shifting to lower temperatures to compensate for decreased $T_{max}$.

*Applying nonlinear averaging to estimate in situ phytoplankton population growth rates*

I estimated time-averaged population growth rates in thermally variable environments for a diverse set of phytoplankton species using nonlinear averaging (Equation 1) and scale transition theory (Appendix B, Equation S5). I estimated TPCs for 89 species by fitting Equation 3 to published phytoplankton growth rates (Thomas et al. 2016) measured in the lab at arrays of constant temperatures using maximum likelihood estimation with the `mle2` function in the `bblme` package in R (Bolker 2017) (Appendix B).

For each of these 89 species, I used historical reconstructed sea surface temperature data to characterize thermal regimes at isolation locations reported in the original studies. For each species’ isolation location, I extracted daily sea surface temperatures (SST) from the closest point in NOAA’s Optimum Interpolation Sea Surface Temperature dataset (OISST), Advanced Very High Resolution Radiometer (AVHRR) and Advanced Microwave Scanning Radiometer on the Earth Observing System (AMSR-E) AVHRR+AMSR, which uses additional data from AMSR-E, available from 2002 to 2011 (Reynolds et al. 2007). This dataset has 0.25° spatial resolution. I calculated the mean and standard deviation of daily sea surface temperatures from
I used daily temperature data because these data reflect diurnal and seasonal variation that is central to understanding patterns of long-term population persistence in phytoplankton.

**Statistical estimation of ‘realized’ TPCs**

I produced two TPCs for each phytoplankton species – one generated assuming temperatures remain constant through time (akin to constant lab conditions) (‘constant’ scenario), and one that incorporates natural patterns of thermal variability from their habitat (‘variable’ scenario). For the ‘constant’ scenario, we fitted Equation 3 to each species’ population growth rate dataset using the methods described above, and estimated a growth rate, \( r \), for the species at the isolation location using \( T = \text{mean annual SST at the isolation location} \). For the ‘variable’ scenario, I estimated in situ growth rates using two approaches: first using Equation 1 where \( T_i \) is daily temperature at the isolation location and where \( f(T) \) is the TPC fit using Equation 3, and second using the scale transition theory (Taylor approximation) approach (Equation S5) where \( f(T) \) is the TPC fit using Equation 3, \( \overline{T} \) and \( \sigma^2_T \) are the mean and standard deviation of daily temperatures over the period 1981-2011. My purpose in using these two approaches was to compare the predictions made with empirical time series of temperature vs. only the mean and standard deviation of the temperature distribution. I present the results from the scale transition theory approach in Appendix B. Then, for each species and isolation location, I applied these approaches over the entire TPC, to generate an expected growth rate at each mean temperature assuming a distribution of temperatures that is identical to the daily temperature distribution observed over the historical time period. To do this, I first generated a synthetic temperature distribution around each mean temperature from -2°C to 40°C by taking the distribution of
temperatures over the historical time series at each isolation location, subtracting the mean, and then adding each temperature from -2°C to 40°C. I then predicted time averaged growth rates as described above (using Equations 1 and S5 in Appendix B). This process yielded a ‘realized’ TPC, which represents expected growth rates given natural patterns of temperature variability. For the ‘constant’ and ‘variable’ scenarios, we compared three attributes of TPCs: \( T_{opt} \), \( T_{min} \) and \( T_{max} \). Given that differences in TPCs based on constant vs variable thermal environments depend on curve shape and temperature variance (Equation S5), I also explored how the discrepancies in estimated critical temperatures and population growth rates at average in situ temperatures depend on the shape of the TPC observed in constant lab conditions. I examined the effects of curve attributes including the skew, using a curve skewness metric developed by (Thomas et al. 2016) (Supplementary Equation 5 in (Thomas et al. 2016)), which standardizes the absolute skewness of the curve by the niche width, \( w \), using OLS regression. All analyses were conducted in R (version 3.4.1, (R Core Team 2017)). All of the data and code for these analyses are available at https://github.com/JoeyBernhardt/thermal-variability.

3.4 Results

1) Does population growth in a thermally variable environment reflect the effects of non-linear averaging over the TPC?

Population growth in fluctuating conditions differed from that in constant thermal conditions over the thermal gradient, and the differences were predicted quantitatively by nonlinear averaging of temporal variation in temperature-dependent performance (95% CI of the growth
rate estimates under fluctuating conditions overlapped with predicted growth rates from Equation 1; orange curve and dashed band in Figure 2B). Consistent with expectations based on Jensen’s inequality and nonlinear averaging, experimental populations of *T. tetrahele* had higher population growth rates under fluctuating temperature conditions compared to constant conditions over accelerating portions of the TPC (i.e. at low mean temperatures; 5°C and 10°C), but lower growth rates under fluctuating temperature conditions compared to constant conditions over decelerating portions of the TPC (i.e. at mean temperatures above 10°C, including 15°C, 24°C and 27°C and 29°C) (Figure 2B). Notably, population growth was lower under fluctuating conditions relative to constant conditions at 24°C, which is close to *T*_{opt} in this population of *T. tetrahele* (Figure 2B). Populations had negative growth rates at 32°C. The shift between positive effects of temperature fluctuations on population growth at low temperatures and negative effects of fluctuations at warmer temperatures aligned with the inflection point of the constant-temperature TPC (16.76 °C, 95% CI: 16.76°C, 16.81°C), providing strong empirical support for nonlinear averaging in predicting population growth in thermally variable environments.

Thermal variation altered estimated parameter values and key features of the realized thermal performance curve. The maximum exponential growth rate (*r*_{max}) was lower under variable conditions than constant conditions, *r*_{max} = 1.54 day^{-1} (95% CI: 1.52 day^{-1}, 1.56 day^{-1}) vs. *r*_{max} = 1.20 day^{-1} (95% CI: 1.15 day^{-1}, 1.25 day^{-1}) (Figure 2A, B). Estimated mean optimal temperatures for growth rate were lower under variable conditions: *T*_{opt} = 24.69°C (95% CI: 24.52°C, 24.88°C) vs. *T*_{opt} = 21.92°C (95% CI 21.48°C, 22.43°C). Maximum mean temperatures for positive growth rates were lower under variable conditions *T*_{max} = 32.39°C
(95% CI: 32.13°C, 32.64°C) vs. \( T_{\text{max}} = 30.31°C \) (95% CI: 29.24°C, 31.97°C). All estimated critical temperatures under fluctuating conditions (\( T_{\text{opt}}, T_{\text{max}}, T_{\text{min}} \)) were quantitatively consistent with theoretical predictions from Equation 1 (i.e. had 95% CI overlapping the predicted values from Equation 1; Figure 2B, C). The range of temperatures associated with positive growth rates, accounting for the freezing point of seawater, i.e. \( T_{\text{max}} - T_{\text{min}} \) was 34.19°C (95% CI: 33.93°C, 34.44°C) under constant conditions and 32.11°C (95% CI: 31.04°C, 33.77°C) under variable conditions. The estimated thermal breadth, \( w \), was also lower under variable conditions, but not statistically distinguishable from constant conditions (i.e. had overlapping 95% CI): \( w = 37.05°C, 95% \text{ CI: 33.57°C, 45.52°C}, \) vs \( w = 41.23°C, 95% \text{ CI: 37.31°C, 47.41°C} \).

2) How different are predicted ‘realized’ TPCs in variable natural environments from predictions based on TPCs generated under constant conditions?

When I estimated the TPCs of the 89 phytoplankton species for constant and varying temperature regimes, I found that for the 90% of species that show negative skew (i.e. mean < median), \( T_{\text{opt}} \) in variable environments is lower than \( T_{\text{opt}} \) in constant environments (Figure 3C), while for the remaining 10% of species which show a positive skew, thermal variability is expected to increase \( T_{\text{opt}} \) relative to \( T_{\text{opt}} \). The magnitude of the difference between \( T_{\text{opt}} \) and \( T_{\text{opt}} \) increases with increasing standard deviation of sea surface temperature and is well explained by curve skew (slope = 85.98, 95% CI: 70.13, 101.82) and the standard deviation of sea surface temperature (slope = -0.32, 95% CI: -0.40, -0.24) (Adjusted \( R^2 = 0.66, F_{2,86} = 85.32, p < 0.001 \)
(Figure 3C). Phytoplankton growth rate estimates that include the effects of thermal variability, \( \bar{r} \), differ from those that do not account for in situ thermal variability, \( r \), (Figure 3D, F).

Generally, predicted growth rates under variable conditions are lower than predicted growth rates assuming constant conditions (i.e. \( \bar{r} - r < 0 \), data points below the line \( y = 0 \) in Figure 3F), however for some species living in regions with thermal regimes typically colder than the species’ \( T_{opt} \), growth rates in these environments can exceed those in constant conditions (\( \bar{r} > r \)). Importantly, the differences between \( r \) and \( \bar{r} \) are greatest for species whose isolation locations have mean temperatures that are close to their \( T_{opt} \). Predicted upper thermal limits for population growth are almost always lower under variable conditions (\( T_{max} < T_{max} \)) (Figure 3E), and the difference between \( T_{max} \) and \( T_{max} \) increases with increasing skewness (positive slope = 53.31, 95% CI: 39.77, 66.85) and standard deviation of SST (negative slope = -0.50, 95% CI: -0.57, -0.43) (Adjusted \( R^2 = 0.77, F_{2,75} = 132.8, p < 0.001 \)).

For all 89 species in the global dataset, the nonlinear averaging approach (presented here) and the scale transition theory approach (Appendix B) resulted in similar ‘realized’ TPCs in variable environments (Figure B4). Predicted critical temperatures, \( r_{max} \) estimates, and relationships shown in Figure 3 were all qualitatively consistent between the two approaches (Figures B4 and B5) indicating that scale transition theory, which makes use of parameters of environmental variation rather than detailed time series, leads to similar predictions in the datasets considered here.
3.5 Discussion

As climate changes worldwide, how temperature affects population growth is a critical link between climate and species persistence in a changing world. One common approach to project population abundance, persistence or fitness under future climate conditions is to apply mathematical curves describing population growth rate over a range of temperatures (a TPC) generated from controlled lab studies (e.g. Deutsch et al. 2008). This approach relies on the assumption that TPCs do not vary systematically with thermal variation. Here I tested this important assumption and found that natural levels of environmental variability systematically change how population growth depends on temperature. In my analysis of globally distributed phytoplankton TPCs, I found that a variable thermal environment reduced critical upper mean temperatures ($T_{max}$) for population persistence by up to 4°C, meaning that population growth in variable conditions was much lower at warmer temperatures than would be predicted based on a TPC generated under constant conditions. This thermal differential is substantial – the 4°C difference in $T_{max}$ is on par with the magnitude of predicted temperature changes over the next 100 years (Frölicher and Paynter 2015), suggesting that projections of TPCs used for future conditions may overestimate population performance in warming climates. Other work has compared acute thermal physiological limits (e.g. $CT_{max}$, $CT_{min}$) to environmental temperatures at range limits to assess relative sensitivities of range edges to warming (e.g. Sunday et al. 2012) yet the underlying nonlinear negatively-skewed thermal performance curve expected for these ectotherms suggests that variability at warm range edges will have a stronger effect on population persistence than variability at cold range edges. Specifically, my findings suggest that approaches based on direct applications of lab-determined critical temperatures may under-
predict range edges at boundaries defined by cold temperatures, and over-predict range edges at boundaries defined by warm temperatures.

I have shown experimentally that realized TPCs in variable environments differ from those in constant environments, and that these differences are predicted qualitatively by Jensen’s inequality (Ruel and Ayres 1999, Denny 2017) and quantitatively from nonlinear time averaging of performance over the TPC (Chesson et al. 2005). Fluctuating temperatures changed several aspects of the ‘realized’ thermal performance curve, including the mean temperature of optimal population growth ($T_{opt}$) and the maximum growth rate ($r_{max}$) – effectively shifting the TPC toward lower temperatures and lower population growth rates overall. Consistent with the argument that ‘suboptimal’ is optimal (Martin and Huey 2008), we show both experimentally (Figure 2B) and theoretically (using empirical TPCs and in situ temperatures; Figure 3D, F) that population growth rates are often lower under variable thermal conditions relative to constant ones, and this negative effect of temperature variation is greatest for populations living close to their thermal optima. However, in contrast to the common assumption that environmental variation is always detrimental for population growth rates (Lande et al. 2003), my results suggest that populations living at mean temperatures in an accelerating part of the TPC will benefit from environmental variation. Indeed, the $T. tetrahele$ isolate used here was collected at a location where mean annual temperatures are far colder than its $T_{opt}$, in the accelerating portion of the negatively skewed TPC (at mean temperature = 6.92°C Reynolds et al. 2007). In this way, when TPCs have accelerating portions at the edges of the thermal niche, thermal variation may allow population persistence in environments that would be too hot or cold under constant conditions.
When I applied nonlinear averaging to estimate the growth rates of globally distributed phytoplankton species, I found that the effect of variability of predicted phytoplankton thermal performance depended strongly on the shape and skew of the TPC and the degree of thermal variability in the oceans from which the phytoplankton originated. Previous approaches have, in the absence of more complete datasets, assumed a certain shape to the reaction norm or TPC (i.e. a Gaussian rise and a parabolic fall; (Vasseur et al. 2014)), thus forcing certain outcomes of variability. Here I used a model that does not prescribe any particular shape (i.e. allows for fully decelerating curves, or curves with accelerating portions, or any combination of decelerating and accelerating portions), thus enabling a more complete exploration of the potential effects of temperature variability on population performance. Importantly, empirical TPCs varied in skew, and whether the TPC was positively- or negatively-skewed determined the direction of the shift between $T_{opt}$ and $\bar{T}_{opt}$. The majority of the curves in the dataset were negatively skewed, and in these cases variability shifted $T_{opt}$ to colder temperatures. Negatively skewed TPCs are widely observed across ectothermic taxa (Dell et al. 2011), suggesting that the direction of the effects of thermal variability observed in my experiment may be general across many ectothermic taxa and ecosystems. Because the shape and skew of the TPC determine performance in variable environments, the mechanisms that determine the shape of the thermal performance curve can have an important influence on the outcome of thermal variability on population persistence. More studies of the diversity of TPC shapes among species will elucidate the extent to which environmental variability increases or decreases performance optima relative to constant lab conditions.
The mathematical tools I applied are generalizable to assessments and projections of biological responses to environmental change and should replace the direct application of TPC parameters based on constant conditions in the lab. In the absence of empirical temperature time series, predictions made based on the mean and standard deviation of the temperature distribution may provide a sufficiently accurate approach. I predicted similar effects of thermal variability on population growth rates when these predictions were made using empirical time series of in situ SST (Equation 1) and when using a Taylor approximation approach from scale transition theory (See Appendix B, Equation S5, and Figures B4-5). Indeed, most of the phytoplankton strains I studied were isolated at locations with thermal regimes corresponding to portions of their TPCs lower in temperature than the highly non-linear temperature ranges near $T_{opt}$.

My results, that performance in fluctuating environments can be predicted from TPCs generated in constant conditions, differ from two previous attempts to predict individual somatic growth rates in fluctuating environments based on TPCs generated in constant conditions (Niehaus et al. 2012, Kingsolver et al. 2015). Previous observations showed that short-term acute responses to diurnal temperature variation were not predictable based on TPCs generated from chronic exposure to constant temperatures over the course of development of an insect (Kingsolver et al. 2015) and amphibian (Niehaus et al. 2012). These contrasting results highlight the importance of the time scale of temperature exposures used to measure and predict performance in constant and fluctuating conditions. Biological responses to temperature, including acclimation and thermal stress are inherently time-dependent and may accrue over the course of development in longer lived species (Schulte et al. 2011, Kingsolver and Buckley 2015, 2017, Kingsolver and Woods 2016), thus precluding the ability of TPCs generated over longer terms (i.e. entire lifespans of
individuals) to predict temperature responses over relatively short time spans (small fractions of
lifespans corresponding to daily temperature variation). In my experiments, using a fast-growing
phytoplankton with short overlapping generations, I maintained the time frames of temperature
exposure comparable under both constant and fluctuating conditions. I used *acute* thermal
performance curves for population growth rates, which integrate responses to temperature over
several generations, generated with a very short acclimation duration at each test temperature, to
predict *acute* responses to thermal variability, also over several generations, thus keeping the
time frames comparable. By measuring population growth over several generations in both
constant and fluctuating conditions, this approach allowed me to avoid mismatches in time scale
and time-dependent effects and instead test the nonlinear effects of temperature variation.

The predictions I made of *in situ* phytoplankton population growth rates should be interpreted as
first-order predictions, which do not incorporate long-term phenotypic responses to thermal
variability. Organisms may be able to acclimatize or adapt to fluctuating conditions over longer-
term exposures (Angilletta 2009), with the potential to alter the shape and limits of the TPC
during the time-course of environmental variability. The global predictions we make here should
be viewed as null models which do not incorporate long-term biological responses to
environmental variability, and should be tested empirically (Estay et al. 2014, Dowd et al. 2015).
To extend my predictions of time-averaged growth rates at the isolation location temperatures to
time-averaged growth rates over the whole thermal niche, i.e. my visualization of a ‘realized
TPC’, I had to assume a particular distribution of temperatures around each hypothetical mean,
and used the variation observed at each isolation location as the residual variation around each
putative mean temperature. This assumption about temperature distributions is a simplification of
real thermal regimes, which likely show more complex patterns of variability and temporal autocorrelation, which can further modify the effects of variability on populations (Gonzalez and Holt 2002). Resource supply is also likely to covary with temperature, potentially altering the outcomes of thermal variability on population growth rate. Nevertheless, even in the simplest scenario of environmental variability, I predict significant changes in realized mean TPC critical temperatures.

Understanding population responses to temperature now and into the future involves understanding biological responses to changes in the full cassette of temperatures experienced – i.e. all the variation. Omitting the effects of environmental variation from population and species distribution models may limit our ability to predict species’ responses, particularly at the extreme edges of their ranges, even if variability patterns remain unchanged. I show that the effects of environmental variation can be predicted based on the shape of the functional relationship between population growth and the environment, adding another tool to the kit for forecasting species’ responses to the environment in a changing world.
3.6 Figures

Figure 3.1 Anatomy of a thermal performance curve.

A) A thermal performance curve and its critical temperatures ($T_{\text{min}}$, $T_{\text{max}}$, $T_{\text{opt}}$) and thermal breadth, ($w$). The critical temperatures are not fitted parameters of the TPC, but are estimated by numerical optimization. This negatively-skewed curve shows an exponential increase typical of processes following an Arrhenius function, with an accelerating region to the left of the inflection point (grey vertical line), followed by a decelerating region to the right of the inflection point. Notice that the accelerating region corresponds to the region with a positive second derivative (B). Predictions for the temperature ranges in which thermal variability is expected to increase and decrease performance relative to constant conditions are shown. Figure adapted from (Dowd et al. 2015), but parameterized with $T. tetrahele$ data from this study.
Figure 3.2 Thermal performance curves for *T. tetrahele* populations growing under constant and variable temperature conditions.

Thermal performance curves for *T. tetrahele* populations growing under constant and variable temperature conditions. A) Exponential growth rates under constant temperature conditions;
green line is the fitted thermal performance curve and green shading corresponds to 95% CI generated from non-parametric bootstrapping. The dashed curve represents predicted growth rate under thermally variable conditions based on nonlinear averaging (Equation 1). Points and error bars are observed mean growth rates generated by estimating exponential growth rates at each mean temperature separately (‘indirect’ approach described in Appendix B; error bars represent 95% CI). B) Exponential growth rates under thermally fluctuating conditions (±5°C); dashed curve is predicted based on panel A and Equation 1, orange line is the fitted thermal performance curve under fluctuating conditions, orange shading corresponds to 95% CI generated from non-parametric bootstrapping. Orange points and error bars are observed mean growth rates in the fluctuating temperature regime (estimated via the ‘indirect’ approach, Appendix B; error bars represent 95% CI). C) Predicted and observed $T_{opt}$ and $T_{max}$ were statistically indistinguishable (predicted: black triangles and 95% CI error bars, observed: orange triangles and 95% CI error bars), and lower than observed $T_{max}$ and $T_{opt}$ in constant conditions (green triangles and 95% CI error bars).
Figure 3.3 Curve skew and environmental variation explain differences between performance under constant and variable conditions.

Curve skew and environmental variability predict differences between performance under constant and variable conditions. A) Diagram showing predicted differences in key features of a thermal performance curve under constant (black line) and variable (grey line) environmental conditions. The distances labeled “C”, “D” and “E” illustrate the distances between curve features, plotted in the panels C-E. B) Map of all phytoplankton isolation locations used in the analysis. The color of the ocean and the points corresponds to standard deviation of daily sea surface temperatures over the time period 1981-2011 (Reynolds et al. 2007). C) The difference between predicted $\bar{T}_{opt}$ and $T_{opt}$ generated under constant lab conditions. D and F) Predicted differences in phytoplankton growth rates that do not incorporate in situ temperature variation ($r$) vs. predicted growth rates based on Equation 1 ($\bar{r}$). E) The difference between $\bar{T}_{max}$ and $T_{max}$. Color coding in panels C, E, and F as in B.
Chapter 4: A trade-off between resource supply and metabolic demand explains the temperature-size rule

4.1 Summary

The temperature-size rule, in which smaller body sizes are observed as temperature increases, is a widely documented pattern among ectotherms. Several competing physiological mechanisms to explain the temperature-size rule have been proposed and supported in different ecological contexts, so it is unclear whether there is a general basis for the temperature-size rule. Beyond the proximate mechanisms, it is necessary to establish the ultimate cause: is the temperature-size rule an inevitable consequence of physiological constraints alone or is it also an adaptive response to current conditions? I address this problem by testing a general model of body size optimization based on energetic requirements and quantifying the fitness consequences of variation in temperature-dependent body size. I test the hypothesis that optimal body size is governed by a trade-off between resource supply and demand by exposing Daphnia pulex to an experimental temperature gradient while holding resource supply constant. I find that increased metabolic demands are associated with smaller adult body sizes under warming, lending strong quantitative support for the existence of a physiological rate-size trade-off shaping plastic responses to temperature. Within a temperature treatment, individuals with smaller adult body sizes have higher reproductive output per unit time, suggesting that, at least at higher temperatures, ‘smaller is better’. Taken together, these results suggest that the temperature-size rule is maintained by physiological and thermodynamic constraints that have important fitness consequences.
4.2 Introduction

The temperature size-rule (TSR), which describes a decrease in body size with increasing temperature is one of the most widely-documented temperature-related patterns among ectotherms; so widely observed as to be dubbed ‘a third universal response to warming’ (Atkinson 1994, Kingsolver and Huey 2008, Gardner et al. 2011). The TSR refers to observed within-species patterns of smaller body sizes at a given developmental stage under warmer rearing conditions (Atkinson 1994). Body size is a fundamental biological trait because it is associated with individual physiology, ecology and fitness (Peters 1986). Body temperature affects biological rates from molecular processes to whole organism metabolism, with consequences for processes ranging from physiological rates to generation time (Gillooly et al. 2001, 2002). Together, temperature and body size play fundamental roles in ecology and evolutionary processes.

Three broadly observed empirical patterns relating temperature, body size, and fitness were summarized by Kingsolver and Huey (2008) as: ‘bigger is better’, ‘hotter makes you smaller’ and ‘hotter is better’. Taken together, these three rules appear to be in conflict and form a life history puzzle that has been the subject of extensive research (Angilletta et al. 2004; Atkinson and Sibly 1997; Hirst and Forster 2013; Sibly and Atkinson 1994). If bigger is better in terms of fitness, but hotter is smaller, suggesting reduced fitness reflecting the positive relationship between fecundity and body size (Kingsolver and Pfennig 2007), then what explains the broad pattern in which organisms are smaller at warmer temperatures (a pattern called the ‘temperature size rule’, or TSR)? At warmer temperatures populations grow exponentially faster than at colder temperatures, suggesting that in terms of population-level fitness metrics, hotter is better (Eppley 1972, Savage et al. 2004). This is surprising, given the general positive correlation
between body size and fecundity, suggesting larger individuals are more fit (e.g., bigger is better). To date, no complete explanation has been found for why the temperature-size rule should be so commonly observed and should persist after initial temperature changes. Is the temperature-size rule a product of physiological and thermodynamic constraints alone (der Have and De Jong 1996, DeLong 2012, Zuo et al. 2012), or is it also maintained by selection on smaller body sizes for the faster population growth rate, rather than the individual fitness gains associated with larger size (Scheiner and Lyman 1991, Walters and Hassall 2006)? Despite extensive empirical documentation of the temperature-size rule among ectotherms (Forster et al. 2012), the extent to which a general mechanism underlies this pattern in a range of ecological contexts remains unresolved, despite extensive treatment of optimal body sizes in life history theories (Stearns 1992, Levitan 2000, Kiflawi 2006).

Body size reflects evolution in environments, through which selection hones the many costs and benefits associated with size and size-related traits. As temperatures increase, nonlinear increases in metabolic rates drive faster somatic growth rates, and for many organisms, faster somatic growth rates are associated with smaller size-at-stage (der Have and De Jong 1996, Ghosh et al. 2013). Whether decreased development time associated with warmer temperatures (Huey and Berrigan 2001) outweighs the reductions in fecundity and survival associated with smaller body sizes (Kingsolver and Pfennig 2007) will determine the net effect of temperature-induced variation in body size on fitness. The physiologically-driven TSR could thus lead to lower fitness due to the lower fecundity of smaller individuals. If true, selection might favor larger body sizes or traits that otherwise increase fitness at higher temperatures. However, if shorter generation
times balance the potential fitness penalty of lower fecundity, then the TSR could be neutral with regard to selection.

As temperatures increase and metabolic demands increase, body size is predicted to decrease due to physiological constraints on resource supply and demand (DeLong 2012). Metabolic demand is expected to increase with temperature proportionally to the temperature dependence of processes underlying growth, maintenance and reproduction (Gillooly et al. 2001, Dell et al. 2011, Pawar et al. 2016). As such, optimal body size, and reduced body size at warmer temperatures, are predicted to arise as a result of physiological compensating mechanisms that balance demand with supply (DeLong 2012). Any deviations from this optimal compensating mechanism will result in body size variation on which selection may act. If variation in body size within a temperature is associated with variation in relative fitness, creating an opportunity for selection, then we can infer that the plastic TSR response may be under selection. Using Daphnia pulex, a clonal model organism for the study of evolutionary trade-offs (Scheiner and Berrigan 1998), I address whether temperature-related increased metabolic demands are the proximate cause of the TSR and how declining body sizes with warming affect fitness. I address two key questions: 1) Does an energetic model for body size optimization explain body size responses to temperature? and 2) What are the potential fitness consequences of variation in temperature-dependent body size? I derived predictions from a general theory for body size that assumes body size is selectively neutral, and then tested the fitness consequences (Figure 1A, B). I experimentally compare the demographic consequences of temperature on fecundity and generation time in genetically distinct Daphnia clones to address the fitness implications of
temperature-induced changes in body size and associated biological rates in terms of an energetic supply-demand model (Figure 1C, D).

4.3 Methods

The supply-demand model for body size

A general mechanistic explanation for body size optimization and its systematic declines with warming, based on an energetic supply-demand model (DeLong 2012), has been met with strong empirical support (DeLong and Hanley 2013). This simple, general model does not include taxon-specific assumptions, and produces quantitative predictions that can be tested in any system. This model proposes that optimal body size should match organismal demand, $D$, for resources with the expected level of per capita resource supply, $S$ (DeLong 2012). The demand curve is written as a power function, $D = b_0 m^b$, where $b_0$ reflects mass-specific demand, $m$ is body mass and $b$ is an allometric scaling exponent that reflects the change in resource use with body size within a species. For consistency with DeLong 2012, I use $b_0$ to denote organismal metabolic rate, not a normalization constant, as is often done in the MST literature. Organisms grow from mass at birth, $m_b$, to asymptotic body size $m_\infty$ or adult body size in species with determinate growth. Optimal body size occurs when $S = D$ and $S = b_0 m_\infty^b$. Solving for $m_\infty$ gives $m_\infty = (S/b_0)^{1/b}$, which shows that resource supply imposes a trade-off between asymptotic body size, $m_\infty$, and mass-specific demand, $b_0$. If resource supply is held constant, then increasing demand, $b_0$, results in a decrease in $m_\infty$. Taking the log of both sides gives,

$$\log(m_\infty) = \frac{1}{b} \log(S) - \frac{1}{b} \log(b_0).$$

(1)
When resource supply, $S$, is held constant, Equation 1 predicts a negative linear relationship between $\log(b_0)$ and $\log(m_\infty)$ with a slope of $-1/b$. Individuals with body sizes that require less than the expected amount of resources should be competitively inferior due to unnecessary limits on biomass production. Individuals with body sizes that require more resources than are available are at a disadvantage because they are taking in less energy than they require. An organism should continue to grow just until it uses as much energy as is available, and no more.

Warmer temperatures, via consistent positive effects on metabolism and processes underlying resource use, growth and reproduction (Gillooly et al. 2001, Dell et al. 2011, Pawar et al. 2016) increase resource demand (Figure 1A). Here I use ‘demand’ to mean the requirement for resources that will be used for growth, maintenance and reproduction. If mass-normalized organismal resource demand increases proportionally with the activation energy ($E_a$) of processes governed by respiration, $b_0 \propto e^{\frac{E_a}{kT}}$, where $T$ is temperature in kelvin and $k$ is Boltzman’s constant, then $\log(m_\infty)$ should scale with temperature as

$$\log(m_\infty) \propto \frac{-E_a}{bkT} \tag{2}$$

As a result, the supply-demand model (Equation 1), which explicitly incorporates resource demand, predicts a decrease in optimal adult body size with warming (Figure 1B). There exists evidence for a size-rate trade-off governing body size responses to temperature in protists (DeLong 2012). The supply-demand model implies that the rate-size trade-off compensating mechanism is fitness maximizing because it underlies efficient resource use and thus increased fitness (Brown et al. 1993), but this has never been tested.
To determine whether an energetic (not adaptive) model of body size optimization is accurate and sufficient to explain organismal responses to temperature, I test the central hypothesis that optimal adult body size for a given phenotype results from a balancing of metabolic demands with available resources (DeLong 2012), and three related predictions.

**Prediction 1.** If body size is governed by a trade-off between resource supply and demand, then the prediction that follows from this is that the association between physiological demand (i.e. growth rate in mg/ind/day) and body size will have a slope of \(-1/b\) (assuming constant resource supply, \(S\)) (Equation 1, Figure 1A).

**Prediction 2.** If increasing metabolic demands underlie the temperature-body size response, then I predict the association between \(\log(m_c)\) and temperature \((1/kT)\) will have a slope of \(-E_a/b\) (Equation 2, Figure 1B). Tests of these predictions (Predictions 1 and 2) can be considered critical tests of the theory, because if these predictions are rejected empirically, then a rate-size trade-off may be insufficient to explain variation in body size associated with temperature.

To assess the fitness consequences of short-term body size shifts with temperature, I quantified temperature responses of several fitness proxies, including clutch size, generation time (time from birth to first reproduction) and the intrinsic rate of increase, \(r\). The intrinsic rate of increase, \(r\), is an appropriate measure of fitness when individuals differ substantially in generation time (i.e. for species that reproduce continuously or multiple times per year such as the *Daphnia* used here) vs. \(R_0\) which more appropriate for species like annual plants (Huey and Berrigan 2001).
The *Daphnia* species I used here is clonal, allowing me to isolate isoclonal lines, thus ensuring genetic variation among replicates.

*Prediction 3.* Since the presence of a rate-size trade-off would indicate an energetic compensating mechanism, then smaller body sizes under warmer conditions should be associated with enhanced fitness (Figure 1C).

*Study organisms and culture conditions*

Mature *Daphnia pulex* females were collected from cattle tanks at the UBC ponds facility in Vancouver, BC. *Daphnia* were housed in the lab under constant temperature (20°C) and light conditions 16:8h light:dark in 200 mL beakers filled with COMBO medium (Kilham et al. 1998) and algal food (*Scenedesmus obliquus*) maintained at saturating conditions (i.e. > 1mg C/L) for three months prior to experiments.

*Determining the mass and temperature dependence of metabolic rate*

I determined the mass and temperature dependence of metabolic rate of *Daphnia pulex* by measuring respiration rates in a sealed glass microplate equipped with oxygen sensor spots in 200ul chambers (Loligo Systems, Denmark) connected to a 24-channel optical fluorescence oxygen system (SDR SensorDish Reader, Presens, Germany). The reader and microplate were placed in a temperature-controlled incubator (Panasonic MIR-154). I rinsed *Daphnia* in COMBO medium, and placed them into wells containing air-saturated COMBO (one individual per well). I removed all bubbles from the wells and sealed them with black plastic caps. After an
acclimation period of one hour, measurements of oxygen concentrations were taken every 15 seconds for one hour. Six blank wells containing COMBO were run at the same time as the *Daphnia*, and the rate of oxygen flux in these wells was subtracted from the experimental wells to account for background microbial respiration. I mass-normalized rates of oxygen consumption by measuring the mass of each individual after being dried for 24 hours at 40°C and weighing it on a microbalance (Mettler Toledo, XPR2U).

*Estimating the temperature dependence of growth rate and body size*

To initiate the experiment, adult females (n = 8 distinct genetic clones) were transferred individually to 200 mL glass beakers filled with COMBO medium and neonates from a single clutch were distributed among each of five temperatures 10, 16, 20, 24 and 27°C to ensure that offspring from a given female were distributed across all temperature treatments. I ensured constant and saturating food supply in the mesocosms by maintaining the concentration of *Scenedesmus obliquus* in the mesocosms at a level well above 2 mg C/L (thus imposing and maintaining a horizontal S curve). Phytoplankton counts were confirmed by taking 0.3 ml samples from the mesocosms weekly and estimating phytoplankton cell abundance and biovolume using a FlowCam (FlowCAM VS Series, Fluid Imaging Technologies) at a flow rate of 0.3 ml/min. Food supplies were replenished as needed by supplementing with *Scenedesmus obliquus* grown in Bold’s Basal Medium (BBM) (Bold 1949) at 20°C. Water changes were carried out at least once per week throughout the experiment to replenish nutrient supply and remove wastes.
I measured body length using a Leica microscope equipped with a camera (Leica MC120-HD; LAS software version 4.4) as the distance from the top of the head to the base of the apical spine. I measured body length of each individual at four time points: as neonates on the first day of the experiment, and then again within 24 hours of each clutch until the third clutch. I converted body length to body mass using an empirically derived length-weight relationship (mass = 0.00402*length ^2.66, \( R^2 = 0.86 \)), which I obtained by measuring the lengths of 50 individuals and then drying them for 24 hours at 40°C and measuring their weights on a microbalance (Mettler Toledo, XPR2U).

Following DeLong and Hanley (2013), I estimated mass-specific growth rate (demand) and asymptotic size using the von Bertalanffy growth model (von Bertalanffy 1960):

$$ l(t) = l_\infty \left(1 - \exp(-kt)\right) $$

where \( l \) is length (mm), \( l_\infty \) is asymptotic body length, and \( k \) is the somatic growth constant (day ^{\text{-1}}). Pilot experiments showed that measuring body sizes at four time points until the third clutch was sufficient to accurately estimate asymptotic body size using the von Bertalanffy model. I use the growth constant, \( k \), as a proxy for mass-specific demand (DeLong and Hanley 2013), because it is a measure of resource allocation to growth, and therefore should be proportional to total resource demand, assuming that allocation ratios do not vary with temperature. The assumption that resources are allocated in fixed proportions to survival, growth and reproduction independent of mass and temperature is an assumption that underlies the size and temperature scaling of life history traits across species (Sibly et al. 2012). I test for deviations from this assumption by comparing the temperature dependence of organismal mass-corrected metabolic rate and the growth constant, \( k \).
Estimating fitness

I checked mesocosms for new clutches daily. Each time a new clutch was born, I transferred the neonates to another 200 mL beaker, supplied with COMBO and Scenedesmus obliquus at saturating food densities. I counted the number of individuals in each clutch three days following birth when the neonates were easily visible to the naked eye. In pilot experiments, I determined that neonate mortality during these three days was negligible by comparing neonate count immediately after birth (i.e. < 8 hours) to the count of neonates three days after birth.

I assessed the effect of temperature-dependent body size on generation time, fecundity and $r$. I measured generation time as the number of days between birth and first reproduction. I measured fecundity by counting the number of offspring produced in each of the first three clutches. I calculated the intrinsic rate of increase, $r$, using Euler’s equation with clutch sizes from the first three clutches.

Data Analysis

I estimated asymptotic body mass and the growth constant by fitting the von Bertalanffy growth model (Equation 3) to time series of body lengths using the nls function in R (version 3.3.0). I tested the effect of temperature on body size and fitness metrics using OLS regression and quantified the relationship between asymptotic body mass and growth rate using reduced major axis regression (RMA) on log transformed data (Equation 1) (Xiao et al. 2011).
4.4 Results

Does the supply-demand model of energetic body size optimization explain body size responses to temperature?

Consistent with the supply-demand model, per capita metabolic rate at 20°C in this population of *Daphnia pulex* increased with body mass allometrically (slope: $b = 1.44$ (95% CI 1.01, 1.89)) (Figure 2). Metabolic rate ($b_0$) and somatic growth rate ($k$), both increased with temperature, with activation energies distinguishable from 0: $E_b = 0.85$ eV (95% CI 0.68, 1.02) and $E_k = 0.67$ eV (95% 0.40, 0.93) (Figure 3). These slopes were statistically indistinguishable, suggesting that resource allocation to growth, vs reproduction or maintenance, was independent of temperature, as assumed. As predicted by the supply-demand model, adult body mass at the timing of each of the first three clutches decreased with warming (Figure 4). The strength of the negative temperature dependence tended to increase over time (at clutch 1, $E_m = 0.23$ (95% CI: 0.12, 0.34), at clutch 2, $E_m = 0.24$ (95% CI: 0.12, 0.36), at clutch 3, $E_m = 0.29$ (95% CI: 0.18, 0.40); non-significant trend over time).

Consistent with predictions of the supply-demand model, I observed a trade-off between body size and somatic growth rate (Prediction 1). Asymptotic body mass, $m_\infty$, was negatively related to the somatic growth constant, $k$. The slope of this relationship, which represents the trade-off between growth rate and body size, was -0.59 (95% CI -0.76, -0.46), which is statistically indistinguishable from the predicted slope (i.e. a slope of $-1/b = -0.69$, based on my empirically derived $b = 1.44$) (Figure 5A). Consistent with Prediction 2, asymptotic body size decreased with warming with a slope of -0.30 (95% CI -0.46, 0.14), a slope shallower yet statistically
indistinguishable from the predicted slope of $-E_{a, \text{demand}}/b = -0.46$ (95% CI -0.21, -0.92) (Figure 5B).

*How does temperature-dependent body size affect potential fitness?*

Two fitness measures varied with temperature in opposing, yet consistent ways. Mass-corrected generation time (time to first clutch) decreased with temperature ($E_a = -0.64$ eV (95% CI: -0.80, -0.47)) (Figure 6A). Mass-corrected intrinsic rate of increase, $r$, increased with temperature with an activation energy of 0.61 eV (95% CI: 0.38, 0.85) (Figure 6B). Fecundity, measured as average clutch size, was unrelated to body size and temperature (Adjusted $R^2 = 0.06$; mass slope = -19.53 (95% CI -73.73, 34.66); temperature slope =0.16 (95% CI -0.006, 0.33). I observed potential fitness benefits of being smaller compared to other individuals at the same temperature as temperature increases (Prediction 3). Intrinsic rate of increase, $r$, increased with temperature, and was negatively related to body mass, within and among temperatures (Figure 6C), meaning that smaller body sizes were associated with higher rates of offspring production ($R^2 = 0.81$, $F_3, 78 = 113.1$, $p < 0.001$).

**4.5 Discussion**

The consistent negative relationship between temperature and body size is one of the most broadly observed patterns among ectotherms. Here I show empirically that a size-growth rate trade-off explains decreased body size under warm conditions. The supply-demand model (DeLong 2012) predicts that optimal body size is achieved when individual demand rates for resources match resource supply rates, leading to a trade-off between somatic growth rate and body size, and ultimately smaller body sizes as resource demands increase relative to supply
rates. By maintaining resource supply constant across temperatures, such that it conformed to the conditions of the supply-demand model, I was able to generate a supply constraint at the individual level. In doing so, I showed that the negative relationship between asymptotic body size \((m_\infty)\) and growth rate \((k)\) predicted by the supply-demand model is in fact a physiological trade-off at the individual level. The negative body size-growth rate relationship does not require other explanations, such as covariation with other factors such as predation risk (Yurista and O’Brien 2001).

Temperature imposes thermodynamic constraints on organismal physiology, and we see these constraints reflected in biological responses including individual growth rate, metabolic rate, generation time and the intrinsic rate of increase, \(r\). Variation in phenotypic responses to these thermodynamic constraints can generate fitness differences. Consistent with theory (Amarasekare and Savage 2011), I find that the strongest temperature effects on fitness were related to generation time (Huey and Berrigan 2001). Clutch size and mortality rates were not consistently related to body size or temperature; instead, these results suggest that temperature effects on fitness are driven by selection on reduced generation time. In contrast to the bigger is better rule, I find that within a given temperature, smaller individuals produced more offspring per unit time, with no apparent increase in mortality. These results suggest that the physiological trade-off between resource supply and metabolic demand that underlies the response of body size to temperature could be associated with increased fitness relative to the organisms’ phenotype in cooler conditions. The strength of the negative relationship between body size and \(r\) was weaker at colder temperatures (Figure 6C), suggesting that when metabolic demand is lower relative to resource supply, growing to larger sizes does not come at a cost to offspring production.
There are many metrics of fitness, and the metric that best approximates fitness will depend on species’ life-history (Huey and Berrigan 2001). The extent to which smaller is better or bigger is better may depend on species’ demography and which metric ($R_0$ vs $r$) best reflects mean fitness in an environment. In the case of species with non-overlapping generations, larger sizes associated with higher fecundity may lead to fitness advantages that do not come with a cost of slower generation time. In contrast to $R_0$, the intrinsic rate of increase, $r$, is defined with respect to time, and thus depends on temperature-driven changes in biological times, perhaps explaining why I observed higher intrinsic rates of increase and smaller body sizes at warmer temperatures. Importantly, here I simplified the selective environment, by excluding predators and competitors. In real selective environments, other factors may favor large body size, including increased predator resistance (Kiflawi 2006) and competitive ability (Bashey 2008). Phenotypic and adaptive responses to predation, including shifts toward larger body size, are commonly observed in *Daphnia* when they are exposed to predation (Spitze 1992, Tollrian 1995), indicating that any temperature-induced shifts in body size will act in the context of other selective pressures in natural environments.

Partitioning fitness into its components, including fecundity, generation time and survivorship demonstrates how temperature effects on life-history traits can affect overall fitness. Here I find that smaller body sizes associated with warmer conditions are explained by a physiological trade-off between resource supply and demand at the individual level, and that temperature constraints on metabolism lead to smaller adult body sizes. Variation in body size that is independent of temperature is associated with shorter generation times, and smaller individuals
generally have faster generation times, leading to greater fitness. This variation in body size that is independent of temperature, and associated with higher relative fitness, creates an opportunity for selection to act.

The effects of the temperature-size rule have broad ecological implications. Body size is a key determinant of food web structure (Woodward et al. 2005), stability of consumer-resource dynamics (DeLong et al. 2015, Osmond et al. 2017) and energy and material cycling (Yvon-Durocher et al. 2011). Understanding the proximate and ultimate causes of temperature-dependent body size is essential to predicting the potential ecological impacts of projected increases in temperature.
4.6 Figures

Figure 4.1 Overview of the supply-demand model, predictions and experimental design.

A) In the supply demand model (DeLong 2012), optimal body size is governed by trade-off between resource supply and demand. Demand (lines labelled D) increases with temperature and body size (m1 and m2 refer to two body sizes where mass of m1 is greater than mass of m2); mass-normalized demand increases with the activation energy \(E_a\) of processes dependent on cellular respiration (slope of demand curve = \(E_a\)). In this figure and throughout the paper, temperature increases from left to right. If resource supply remains constant across temperatures (i.e. horizontal S curve), then as demand increases with temperature, asymptotic body size will decrease. B) As predicted by the supply-demand model, asymptotic body size decreases with temperature with a slope of \(-E_a/b\), where \(b\) is the allometric scaling exponent for the relationship between organismal metabolic rate and body mass. C) The fitness consequences of temperature-dependent body size will depend on the relative influences of temperature-induced changes on generation time, fecundity, and mortality rate on fitness. Temperature will be associated with increased fitness if shorter generation time outweighs the influence of decreased fecundity or increased mortality on fitness. Alternatively, fitness may decrease with temperature if decreased fecundity or increased mortality outweigh the positive effects of shortened generation times on fitness. D) Experimental design; body size was measured at birth and each of the first three
clutches. Fitness metrics were estimated as clutch size, generation time and mortality rates (see Methods for more details).
Figure 4.2 Whole organism metabolic rate increases with body size for adult Daphnia, 20°C.

The slope of the relationship between organismal demand (metabolic rate) and body size (mass) is the allometric scaling exponent ($b$) used in the supply-demand model (Equation 1; Figure 1B).
Demand increases with temperature for two measures of demand: A) growth constant, \( k \), increases with an activation energy of 0.67 eV (95% CI 0.40, 0.93) and B) mass-corrected metabolic rate increases with an activation energy of 0.85 eV (95% CI 0.68, 1.02).
Figure 4.4 Body size decreases with warming over the course of development, and the negative slope of this relationship increases over time.

A) $E_a = -0.23$ (95% CI: -0.12, -0.34), B) $E_a = -0.24$ (95% CI: -0.12, -0.36), C) $E_a = -0.29$ (95% CI: -0.18, -0.40).
Figure 4.5 Test of the supply-demand model.

A) Body size declines with increasing resource demand (growth constant, \( k \)). Predicted slope (i.e. slope = -1/b) is shown in the black dashed line, 95% CI on the prediction are shown in grey dashed lines. B) Following predictions based on increasing resource demand with increasing temperature (Figure 1B; predicted slope = -Ea/b, black dashed line in this figure), body size declines with increasing temperature (grey dashed lines are the 95% CI on the predicted slope).
Figure 4.6 In terms of several estimates of fitness, hotter is better and smaller is better in *Daphnia pulex*. 
A) Mass normalized generation time decreases with a slope of -0.62 eV (95% CI: -0.78, -0.45).
B) Mass normalized intrinsic rate of increase, $r$, increases with temperature with an activation energy of 0.60 eV (95% CI: 0.46, 0.74). C) Intrinsic rate of increase decreases with increasing body size within and among temperatures.
Chapter 5: Aquatic biodiversity enhances nutrition benefits to humans

5.1 Summary

For many people, food and nutrition security is a benefit provided by aquatic ecosystems. Amid increasing evidence that biodiversity enhances ecosystem function and services, we still do not know whether biodiversity provides the multidimensional benefits necessary for food security. I found that biodiversity enhances human nutritional benefits, based on our analysis of micronutrient concentrations in 430 edible aquatic species. Distinct and complementary seafood species micronutrient profiles are related to ecological functional traits including body size. I show that, surprisingly, nutritional metrics that capture multiple nutrients essential for human wellbeing depend even more strongly on biodiversity than ecological measures of function such as biomass or productivity. I provide the first direct link between multifunctional benefits of biodiversity and an ecosystem service underpinning human wellbeing, directly linking biodiversity loss with human health at local and global scales. Extending the multifunctional benefits of biodiversity to human wellbeing via nutrition underscores the need to minimize biodiversity loss for the benefits of humanity.

5.2 Introduction

Obtaining sufficient and nutritious food is one of the greatest challenges facing humanity (Golden et al. 2016). A nutritionally sufficient food supply meets multiple nutritional targets for a range of essential macro- and micronutrients simultaneously (Latham 1997). Wild-caught marine and freshwater finfish and invertebrates (hereafter ‘seafood’) are rich and unique sources
of essential micronutrients, such as vitamins, minerals and fatty acids not found in other foods. Nutritionally complete diets require diverse food sources (Lachat et al. 2018) yet the role of biodiversity in providing nutritional security from seafood remains poorly understood (Penafiel et al. 2011). Given the importance of micronutrients to a healthy human diet, and the reliance of many populations on seafood for these benefits, it is imperative that we address this challenge in a time of global biodiversity change (Elahi et al. 2015, Magurran et al. 2015).

Biodiversity enhances ecosystem functions and services because species differ in functional traits or attributes (Balvanera et al. 2014, Isbell et al. 2017, Duffy et al. 2017). Species losses and range shifts due to climate change, harvesting and other human activities alter aquatic biodiversity locally and globally (Elahi et al. 2015), with consequences for ecosystem functions (O’connor et al. 2017, Isbell et al. 2017). Human nutritional benefits from seafood are an important ecosystem service provided by aquatic biodiversity. If aquatic species differ in nutritional profiles as defined by human nutritional needs, the importance of biodiversity for food security depends on exactly how species’ multi-nutrient profiles vary. Specifically, a ‘biodiversity effect’ of nutritional benefits requires that some seafood species contain high concentrations of some micronutrients while other species contain high concentrations of different micronutrients, creating a complementary distribution of micronutrients across species. Ecosystem services that reflect multiple ecosystem functions are believed to be most vulnerable to negative consequences of biodiversity loss (Byrnes et al. 2014, Lefcheck et al. 2015).
I tested the hypothesis that aquatic biodiversity confers nutritional benefits through complementarity in nutrient concentrations among species. I tested whether 1) biodiversity of seafood supply enhances nutritional diversity \((N_D)\) in seafood diets and 2) biodiversity increases the nutritional content of an edible portion of seafood, thereby improving the efficiency \((N_E)\) with which seafood consumers reach nutritional targets (Figure 1). I predicted that increased species richness in seafood diets yields increased nutritional benefits, and that variation in nutrient concentrations among species is related to species’ ecological traits. In a global analysis, I considered provision of nutritional benefits to human consumers accessing worldwide seafood markets. I then tested whether seafood biodiversity promotes human health at local scales by providing multiple essential nutrients in fourteen traditional indigenous diets in North America. I quantified variation in nutrient concentrations in edible tissues of 430 commonly consumed aquatic species in the global species pool, and 25 – 57 species in fourteen local dietary species pools. This represents the first extension of biodiversity - ecosystem functioning (BEF) theory to a multivariate service defined by the human beneficiary of a service that is directly relevant to human health.

### 5.3 Methods

*Quantifying nutritional value in terms of human health benefits*

I characterized an aquatic species’ nutritional profile by drawing on two well-established nutritional metrics: nutrient concentration (nutrient content/100g edible portion) and Dietary Reference Intake (DRI). DRIs are developed following health guidelines, and quantify the recommended amount of a particular nutrient required to maintain health (IOM 2012). The DRI
used here is the Recommended Dietary Allowance, established by the Food and Nutrition Board of the United States Institute of Medicine, which is daily intake level of a nutrient needed to meet the requirements of 97–98% of healthy adults (males and females above age 19) (IOM 2012). I defined the nutritional value of a fish species for each nutrient in terms of the nutrient content in an edible portion relative to DRI. For some aquatic species, micronutrients are in such high concentrations in edible tissues that a single 100g portion contains the entire dietary reference intake (DRI) for that nutrient, while edible tissues from other species provide only small fractions of the DRI. I considered thresholds of nutritional benefit (function) between 1% and 100% of DRI, although we focus on 10% of DRI since it is a minimum threshold for a food to be considered of nutritional benefit (IOM 2012). I defined a DRI target as 10% of DRI for a given nutrient. I characterized nutritional profiles in two ways: first, in terms of concentrations of each nutrient (single nutrient profile), and secondly, in terms of multiple nutrients simultaneously (i.e. multi-nutrient profile).

Defining nutritional benefits

I quantified the effect of biodiversity on nutritional benefits in two ways (Figure 1): 1) nutritional efficiency, $N_E$, which quantifies that amount of tissue, in grams, required to reach a given number of DRI targets simultaneously and 2) nutritional diversity, $N_D$, which quantifies the number of distinct DRI thresholds met in a standard 100g edible portion. Nutritional benefit increases with decreasing values of $N_E$, since $N_E$ quantifies the grams required to reach DRI
targets, and fewer grams required is better from the perspective of human nutrition. I quantified $N_D$ in an arbitrary daily diet, assuming that the seafood diet contains 100g of seafood per day.

*Literature search and data collection*

I assembled a dataset of published nutrient concentrations in edible portions of 430 aquatic species. I analyzed tissue concentrations of nutrients for which DRI standards exist and that are implicated in a range of biologically important processes that affect organismal growth and reproduction, and therefore may potentially relate ecological function with human nutritional wellbeing. I examined macronutrients including protein and fat, as well as five micronutrients: the polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), metals beneficial at low concentrations but toxic at high concentrations (zinc and iron), and one beneficial mineral (calcium). I aimed to include as many marine and freshwater species as possible covering a wide geographic extent. I searched peer-reviewed literature for analytical food composition values as well as the Food and Agriculture Organization’s Global Food Composition Database for Fish and Shellfish (Food and Agriculture Organization of the United Nations 2016). For finfish, I restricted our analysis to include only edible portions of wild, raw fish (excluding prepared or farmed seafood items). I included both farmed and wild mollusc species because molluse farming does not typically involve additional food inputs, which could influence tissue nutrient composition. For each species, I noted which body parts are included in the edible portion and season of collection. For each sample, I noted the location of origin (e.g. latitude and longitude). To address inconsistencies in fatty acid data reporting, I standardized fatty acid measurements using the fatty acid conversion factors proposed by Nowak et al. 2014 (Nowak et al. 2014). When there were multiple observations available for a single species, I
averaged nutrient concentrations across the observations. I did not include data from national food composition tables because these data usually report seafood data with a generic food description, which does not allow for a clear description of which fish tissues are included in the edible portion. For each species with nutritional data, we collected ecological trait information from FishBase (Froese 2017) and SeaLifeBase (Palomares 2017). I included body size (maximum length), fractional trophic position, temperature preference (using latitude as a proxy), habitat preference, and feeding mode.

Statistical analyses and hypothesis testing

Hypothesis 1: Biodiversity enhances nutritional benefits

I tested the effect of species diversity (quantified as the number of species, or species richness, S) on nutritional diversity and efficiency by randomly assembling diets from the global seafood species pool, keeping the portion size constant (100 grams) across all levels of diversity. In analyses using the global dataset (430 species), I assumed that human populations have access to the entire global species pool and choose species at random. Though this assumption certainly ignores economic, social and cultural factors that affect which species people consume, in the absence of detailed diet information for the majority of the world’s populations this is a necessary assumption. To assess potential effects of biodiversity on nutritional benefits for populations that consume seafood locally and not as part of the global seafood market, I sampled diets from species contained within traditional diets in fourteen indigenous cultures in North America. I used species lists for local diets that were obtained from an ethnographic database of
traditional animal foods of indigenous peoples of northern North America, and only included species that were harvested wild and whose nutrient compositions where analyzed when raw (Table C7, Kuhnlein and Humphries 2017). To avoid confounding differences in biodiversity effects at global and local scales with the sizes of the species pools at each of these scales, I matched the size of the global species pool to the average size of the local species pools (40 species).

I calculated two metrics of nutritional benefits for hypothetical diets comprising species drawn randomly from either the global dataset (global diet) or local datasets (local diets):

1) **Nutritional efficiency** ($N_E$). I tested the hypothesis that complementarity in nutrient concentrations among species enhances $N_E$ by estimating the effect of species richness on $N_E$ (the ‘biodiversity effect’). To estimate the ‘biodiversity effect’ on $N_E$, I took an approach to modeling dietary species composition that is analogous to a biodiversity-ecosystem function experiment with a replacement design, where species abundances decline proportionally as species richness increases. From the global species pool, I sampled ten species at random and then assembled seafood diets from all possible combinations of these ten randomly chosen species at 10 levels of species richness (1-10). I repeated this process of sampling ten species from the global species pool and then assembling all possible diets 1000 times. For each combination of species in each dietary diversity level (1-10 species), I calculated the number of grams required to reach a given DRI target (either: one of six possible nutrient targets individually, or five micronutrient (calcium, iron, zinc, EPA and DHA) targets simultaneously). I quantified the effect of species
richness in a diet on nutritional efficiency, $N_E$, by fitting a power function to these bootstrapped nutritional efficiency estimates:

$$N_E = aS^b$$  \hspace{1cm} (1)$$

where the parameter $b$ describes the relationship between a change in species richness, $S$, and a change in $N_E$, and $a$ is a constant (g). Since $N_E$ is measured in grams required to reach a given DRI target, and fewer grams required is better from the perspective of human nutrition, then a benefit of biodiversity would be reflected in a negative $b$ (i.e. $N_E$, measured in grams of tissue required, decreases with species richness). For each nutrient individually, and for all five micronutrients together, we estimated the exponent parameter, $b$, using non-linear regression using the `nls.LM` function in the `minpack.lm` package in R (Elzhov et al. 2016). To quantify uncertainty in parameter estimates associated with sampling from the pool of observed nutrient content values, I calculated bootstrapped confidence intervals using non-parametric bootstrapping of mean centered-residuals using the `nlsBoot` function in the R package `nlstools` (Baty et al. 2015).

2) Nutritional diversity ($N_D$). To test the hypothesis that complementarity in nutrient concentrations among species increases nutritional benefits by increasing the number of distinct DRI targets in a 100g portion, I constructed dietary reference intake (DRI) target accumulation curves. These are analogous to species accumulation curves used in ecological studies to assess patterns of beta-diversity, or species turnover, in ecological community composition data. I assessed turnover of nutrients content in edible tissues among fish species. Each fish species is
associated with a set of 0s and 1s corresponding to whether or not it achieves a 10% DRI for each of five micronutrients (equivalent to a species presence-absence matrix in community composition data), sampled with replacement 1000 times. This approach allowed us to explore how likely it would be for human diets containing different numbers of fish species to reach a given number of micronutrient DRI targets ($N_D$ ranges between 0 and 5), assuming that fish species were included in the human diet at random. I quantified the effect of biodiversity on nutritional diversity, $N_D$, by fitting a power function,

$$N_D = aS^b$$  \hspace{1cm} (2)

where the parameter $b$ describes the relationship between a change in species richness, $S$, and a change in $N_D$ (i.e. the number of DRI targets reached per average 100g portion), and $a$ is a constant.

For both $N_E$ and $N_D$, we tested the hypothesis that biodiversity enhances nutritional benefits by assessing whether the estimate of the scaling exponent, $b$, had confidence intervals not overlapping zero. I concluded that biodiversity enhanced nutritional benefits if $b$ for $N_E$ was negative and $b$ for $N_D$ was positive.

I tested the hypothesis that nutritional functional diversity drives positive biodiversity effects by testing for a positive association between nutritional functional diversity and the biodiversity scaling exponent, $b$. I hypothesized that nutritional functional diversity would be higher at the global scale than the local scale, because the global species pool contains more ecological and
biogeographic diversity. To assess levels of nutritional functional diversity among species, we calculated functional diversity (FD) (Petchey and Gaston 2002). FD is based on an assessment of the entire functional diversity of a group represented as a functional dendrogram, and FD allows estimation of complementarity among species’ nutrient concentrations (i.e. nutritional functional traits) using the dendrogram. I treated the concentration of each micronutrient (calcium, iron, zinc, EPA and DHA) as a functional trait. I also quantified functional evenness metric ($FEve$) using the FD package in R, which normally quantifies the evenness of abundance in a functional trait space. Here, I used $FEve$ to quantify the evenness in concentration of nutrients across species (Villéger et al. 2008). To compare $FD$ and $FEve$ at the global and local scales, I first subsampled 40 species (the average species pool at the local scale) from the global pool, then calculated the functional diversity metrics on the subsample, and repeated this process 1000 times. Using this same approach, I calculated levels of ‘expected’ $FD$ and $FEve$ for each local diet by choosing random subsets of the global pool with sample size equal to the species pool in each local diet, and repeated this process 1000 times (Figure C5).

Hypothesis 2: Diversity in nutrient concentrations of the edible portion is related to ecological diversity estimated as species’ ecological traits.

I tested the hypothesis that nutrient concentrations are related to species’ ecological traits in two ways: 1) testing whether multi-nutrient profiles (i.e. concentrations of all five micronutrients) differ among major phylogenetic groups and 2) whether differences in single nutrient concentrations differ with species ecological traits. I examined variation in multi-nutrient profiles
among seafood species using the vegan package in R (Oksanen et al. 2013). I ln transformed nutrient concentration data to achieve normality. Differences in multi-nutrient profiles were visualized through non-metric dimensional scaling (NMDS) using the metaMDS function. The ordination ran for 1000 iterations, and the stress score of 0.032 for the final solution was sufficiently low to enable reliable interpretation in the two dimensions (Legendre and Legendre 1998). First, I tested the hypothesis that major phylogenetic groups correlated with functional differences in life history, resource use and ecology (i.e. finfish, mollusc, and crustacean) differ in their multi-nutrient profiles via permutational multivariate ANOVA (PERMANOVA) using the adonis function (999 permutations) based on Bray-Curtis dissimilarity matrices. I used an overall (three-way) PERMANOVA first to investigate phylogenetic group effects on nutrient profile of a species’ edible tissue.

To test for associations between species’ ecological functional traits and their nutrient concentrations, I modeled the relationship between traits and ln(nutrient concentration) with linear regression. The full model included the entire set of trait predictors as fixed effects and a random effect term for each study $j$ capturing systematic variation among studies in terms of how nutrient concentrations were estimated:

$$\ln(\text{nutrient}) = \beta_0 + \beta_1*\ln(\text{body size}) + \beta_2*\text{latitude} + \beta_3*\text{trophic position} + \beta_4*\text{habitat} + \beta_5*\text{feeding mode} + \beta_6*\text{diet breadth} + \mu_j + \epsilon_i$$

I created models from subsets of the full model that represented hypotheses based on the known physiological roles of micronutrients and their relationships to our set of predictors (Tables C2-
C6, C9). To avoid issues associated with multicollinearity of predictor variables, I excluded other possible variables if they were highly correlated (i.e. correlation coefficient $> 0.6$). I identified the best subset of models using the Akaike Information Criterion, adjusted for small sample sizes (AICc). We used AICc, $\hat{\Delta}$aic and Akaike weights ($w$) to compare models. I ranked models based on $w$, and selected the set of models that produced a cumulative $w > 0.95$, meaning that we are 95% confident that the chosen set includes the best model (Burnham and Anderson 2002). In cases where I could not obtain measurements of all traits for all species, I performed model selection on reduced datasets without missing values.

**Uncertainties:**

There are several sources of uncertainty in my analyses. First, there are substantial sources of uncertainty in food composition estimates. The data in my dataset meet international standards for data quality and standardization, meaning that I followed guidelines for checking food composition data and converting units, denominators and expressions (Food and Agriculture Organization/ International Network of Food Data Systems (INFOODS) 2012)). Still, tissue concentrations may vary depending on analytical techniques, labs, season, diet of the animal, life stage etc. Some of these sources of uncertainty (e.g. differences in analytical techniques) are unavoidable consequences of synthesizing previously published data collected across many labs. I assumed that these uncertainties in the data were randomly distributed over our geographically and taxonomically diverse dataset. Further uncertainty is associated with how well my set of 430 species represents the global pool of seafood consumed. I do not know whether our sample is random or biased, though I can say that our dataset includes 41 of the 67 most consumed species
worldwide (as determined by FAO production volumes (Food and Agriculture Organization 2013), species with capture production of 150 000 tonnes or more, after removing species for which the majority of production volume is diverted to fish meal and oil (Cashion et al. 2017), Table C8). A remaining source of variation among samples is likely due to natural sources of variation associated with seasonal and other sources of temporal variability, which I consider to be an important component of biodiversity. For the Nutritional Diversity and Nutritional Efficiency analyses, these uncertainties were not modeled. For the ecological trait analyses, the uncertainty in observation precision was modeled as normally distributed error term, $\epsilon$, at the species level.

To account for model uncertainty in the ecological trait correlation analyses, I performed model averaging of coefficients in all models with $\Delta$AIC < 2 ($\Delta$AIC = AIC$_i$ - AIC$_{min}$), and included zeros as coefficients when variables did not enter a given model (Burnham and Anderson 2002). I conducted our model selection and averaging analyses with the MuMIn package (Barton 2013) and all other analyses in R version 3.3.2 (R Core Team 2017).

5.4 Results and discussion

I found that seafood biodiversity not only enhances nutritional benefits for consumers selecting seafood from species included in our global dataset, but it is essential to meeting nutritional targets. Species in the global dataset differed substantially in micronutrient concentrations, but not protein concentrations, relative to dietary reference intake (DRI) targets (Fig 2; ln(protein) geometric coefficient of variation (geometric CV) = 0.03 vs. micronutrient geometric CVs: ln(iron) = 3.97, ln(calcium) = 3.25, ln(EPA) = 2.52, ln(zinc) = 2.10, ln(DHA) = 1.70). When I
considered each nutrient separately, I found that fewer than half the species reached an arbitrary single-nutrient threshold of 10% of the daily DRI target (IOM 2012) for calcium, iron and the essential fatty acid EPA in a standard 100g portion of a single species (Table C1). As species richness of diets increased, 10% DRI for any micronutrient was achieved with less total seafood intake (Figure 3A, $b < 0$ for every micronutrient: calcium -0.43 (95% CI -0.47, -0.40), iron -0.40 (95% CI -0.43, -0.36), zinc -0.21 (95% CI -0.21, -0.23), EPA -0.25 (95% CI -0.26, -0.24) and DHA -0.21 (95% CI -0.21, -0.20), meaning that increasing species richness lead to enhanced nutritional efficiency ($N_E$). All species reached the protein DRI target, and there was no benefit of seafood diversity for protein (Figure 3A, $b = 0.0092$ 95% CI 0.0086, 0.010).

I then considered the accumulation of multiple nutrients together simultaneously with seafood species richness. I treated each nutrient concentration relative to 10% DRI as one ecosystem function. Consistent with biodiversity-ecosystem functioning theory, we found that in the case of a multifunctional metric of an ecosystem service defined from the human perspective (i.e. multiple micronutrient targets reached simultaneously), biodiversity benefits for the multifunctional service are greater than for individual functions ($b$ for all five micronutrients simultaneously = -0.49 (95% CI -0.50, -0.48) vs. single nutrients $b$ range from -0.43 (95% CI -0.47, -0.40) for calcium to -0.21 for EPA (95% CI -0.21, -0.20)) that comprise the ecosystem service (Figure 3A). Increasing species diversity in a hypothetical diet from one to five species allows consumers to meet 10% of DRI for five essential microelements and fatty acids simultaneously more than twice as efficiently (i.e. a median of 485.83g of tissue required with one species vs. median of 216.96g of tissue required for five species) (Figure 3B, C). Positive
biodiversity effects persisted even when total weight of seafood tissue intake remained constant, so that increases in nutritional benefits were not confounded with simply eating more seafood (Fig 3D). When tissue intake was limited to 100g per day, more diverse diets reached more nutritional targets (higher $N_D$) per serving than diets comprising fewer species ($b = 0.21$ (95% CI: 0.18-0.24) Figure 3D).

Despite recent claims that multifunctionality is not enhanced by biodiversity (Gamfeldt and Roger 2017), here I show that when function thresholds are grounded *a priori* in multivariate metrics meaningful for human wellbeing such as DRI, diversity enhances multifunctionality. These findings are robust to multiple DRI threshold levels (Figure C1), and the biodiversity effect is strongest at a threshold of approximately 28% of DRI (Figure C1). More generally, ecosystem service benefits, as defined in metrics of human wellbeing rather than the traits of the species pool under consideration, typically are produced by several underlying ecosystem functions (Manning et al. 2018). The strong effects of diversity on multifunctional benefits observed here may also apply to relationships between diversity and other services e.g., desired filtration rates of pollutants in wetlands (Boyer and Polasky 2004), or desired pest consumption rates in agricultural systems (Karp et al. 2013).

Consistent with the positive biodiversity effects I observed when assuming consumers have access to global seafood markets, I also found benefits of seafood diversity locally. I analyzed the effects of biodiversity in fourteen traditional indigenous North American diets and found a consistent, positive effect of biodiversity on $N_D$ and $N_E$, although the magnitude of the
biodiversity effect was generally lower at the local scale than the global scale (Figure 3C-D, Figure C2, C5) ($N_D$ global $b = 0.21$ (95% CI 0.18, 0.24) vs mean local $b = 0.14 \pm 0.0083$ S.E. and $N_E$ global $b = -0.50$ (95% CI -0.52, -0.47) vs mean local $b = -0.16 \pm 0.0091$ S.E.). This finding is consistent with lower nutritional functional diversity (mean local $FD = 2.77 \pm 0.17$ S.E. vs. global $FD = 3.87 \pm 0.0096$ S.E.) and higher nutritional functional evenness in local diets (mean local $FEve = 0.76 \pm 0.01$ S.E. vs. global $FEve = 0.71 \pm 0.0018$ S.E.) (Figure C2, C5), suggesting that functional consequences of changes to diversity in local seafood diets may be buffered by higher redundancy among species. Given increasing trends towards homogenization of the global food supply (Khoury et al. 2014) including aquaculture (Duarte et al. 2009, Bostock et al. 2010), this local-scale finding highlights the importance of local species diversity in the diets of vulnerable populations (Bogard et al. 2017).

Substantial variation in nutrient concentrations in edible portions among species can be explained partly by major ecological attributes and traits: taxonomic group, latitude, body size, diet breadth and feeding habits (Tables C2C-S6). Finfish, crustaceans and molluscs differed significantly in their multi-nutrient profiles (PERMANOVA, $F_{2,103} = 3.429$, $p = 0.006$). Among finfish, concentrations of calcium, iron and zinc in edible tissue decreased with increasing body size (Figure 4, negative slopes, $p < 0.01$, Tables S2-S6). Variation in protein and fat was poorly explained by species’ ecological traits (Marginal $R^2 = 0.023, 0.09$, for protein and fat). In addition to ecological traits, finfish species that are eaten whole, or whose edible portions include organs such as skin, liver or bones, have higher nutrient concentrations in the edible portion than those whose edible portions are restricted to muscle tissue ($R^2 = 0.60, F_{5,251} = 76.24$, $p < 0.01$; Figure C3). Nutrient concentrations were typically weakly negatively correlated or uncorrelated
with each other among species (Figure C4), allowing complementarity among species to increase nutritional benefits.

Maintaining the diversity of global fisheries is important for ensuring food and nutrition security. A diverse seafood species pool feeds not only local communities but also seafood markets worldwide, and aquatic species contain micronutrients not found in other foods. Many of the most nutritionally vulnerable populations – those that are deficient in essential micronutrients during particularly sensitive stages of life (i.e. pregnancy, breastfeeding and childhood) may rely on local ecosystems to meet their nutritional demands (Kawarazuka and Béné 2010, Bogard et al. 2015, Golden et al. 2016). These populations may have access to a limited amount of locally available fish tissue each day or to fish from a subset of habitat types, suggesting that for these populations nutritional efficiency may be particularly important. In tropical regions, fish diversity in coastal regions has plummeted in recent decades (Jones et al. 2004) characterized by two-fold declines in body sizes of fish (Cheung et al. 2013, Molinos et al. 2016). These regions are also regions of high nutritional vulnerability and reliance on locally harvested seafood (Allison et al. 2009). However, as the seafood trade becomes increasingly global (Gephart and Pace 2015, Watson et al. 2016), seafood-derived nutrition available to consumers participating in the global market may be related to globally harvested seafood biodiversity. As a result, changes to local biodiversity and resultant impacts on human nutrition may be buffered by access to global seafood markets. Together, my results suggest that in the context of global change, understanding and protecting the potential for nature to support diverse and productive aquatic ecosystems has direct and immediate benefits to humanity.
Aquatic biodiversity enhances human nutritional benefits and human wellbeing. Aquatic biodiversity increases human well-being because edible species have distinct and complementary multi-nutrient profiles (A) and differ in mean micro- and macronutrient content (shown here relative to 10% and 25% thresholds of daily reference intake (DRI) guidelines for representative finfish (*Abramis brama, Mullus surmuletus*), mollusc (*Mytilus galloprovincialis*) and crustacean species (*Nephrops norvegicus*). Biodiversity – ecosystem functioning theory predicts that dietary nutritional benefits, including nutritional diversity (*N_D*; i, iii) and nutritional efficiency (*N_E*; ii, iv) (B, E) increase as grams required to achieve DRIs decrease with dietary species richness (B, E). Seafood consumers with limited access to seafood each day may not reach DRI targets if diets are low in diversity (F vs C).
Variation in nutrient concentrations differs among taxonomic groups. (A) Frequency of reported protein, fat and micronutrient (including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA)) content in 100 g of the edible portion of 430 seafood species (references in Dataset S1). (B) Proportion of species, and number shown on each bar, with available data that reach 10% of RDI targets for any one, two or up to five of the micronutrients examined here.
Figure 5.3 Species diversity enhances nutritional benefits through portfolio effects.

A) Seafood species richness increases the efficiency (grams of seafood consumed to meet nutritional threshold) with which humans can meet 10% of DRI for each of five micronutrients (points are median values for calcium, iron, zinc, EPA, DHA and protein) as well as for five micronutrients simultaneously (purple line labeled ‘5 Micronutrients’); estimates for the $b$ parameter (inset) describe the strength of the biodiversity effect. B) Increasing dietary species richness sourced from global seafood markets increases the efficiency with which five nutritional targets are reached; solid circles are median values, as plotted in the purple line in A. C) Increasing species richness increases the efficiency with which five nutritional targets are reached; shaded areas are 95% confidence intervals about the mean; black lines are for each of fourteen traditional diets in North America, green line is for a diet sourced from the global seafood market. D) Species richness enhances the number of distinct DRI targets met in a 100g portion; color coding as in C.
Figure 5.4 Micronutrient concentrations are significantly negatively related to body size.

Micronutrient concentrations are significantly negatively related to body size in finfish. Negative slopes: calcium: -0.26 (95% CI -0.38, -0.14, \( n = 174 \)), iron: -0.16 (95% CI -0.30, -0.02, \( n = 181 \)), zinc: -0.16 (95% CI -0.30, -0.03, \( n = 161 \)).
Chapter 6: Conclusions

I have tested and extended a general theoretical framework, metabolic scaling theory (MST), to address longstanding questions about the abundance and distribution of life on Earth. What relationships between individual performance and the abiotic environment determine the characteristics of individuals, population growth rate and persistence? These questions are not new (Andrewartha and Birch 1954, Brown 1995). My approach has been to break down these large questions into a set of testable hypotheses within the context of MST, perform critical tests of theoretical predictions and use different lines of evidence to piece together what determines patterns of abundance and distribution. I have drawn inspiration from widely observed macroecological patterns, such as the temperature-size rule (TSR), and used manipulative experiments to reveal their mechanistic underpinnings. I have focused on the links between processes operating at the individual and population levels because these links are the least well understood in the context of MST and yet critical to advancing a unified theory of ecology. Finally, I have considered how aspects of biodiversity, including the diversity of species, traits and functional types in an ecosystem are related to benefits to these systems provide to people.

6.1 Major findings

In Chapter 2, I showed that the temperature-dependence of population dynamics could be predicted from the temperature-dependence of individual metabolism, thus lending strong support for the role of energetic constraints in governing population abundance. Population growth rates underlie a wide range of ecological and evolutionary processes (Lawson et al. 2015)
including phenotypic adaptation (Roughgarden 1979), population persistence (Ovaskainen and Meerson 2010) and range expansions and contractions (Bennie et al. 2013). Identifying an energetic basis for demography may therefore offer a useful way forward in the effort to develop a coherent understanding of current and future patterns of species distributions and abundance.

Thermal performance curves, which describe the relationship between individual or population performance and temperature, are widely used in projections of species distributions under future climate scenarios (Deutsch et al. 2008, Sunday et al. 2012, Thomas et al. 2012). These relationships are an essential component of our ability to predict and forecast the ecological effects of global change. And yet, these predictions are often based on the assumption that performance under constant lab conditions is analogous to performance under naturally variable climate regimes (Deutsch et al. 2008, Sunday et al. 2013, Thomas et al. 2012). In Chapter 3, I showed experimentally that while the effects of thermal variation on population growth are highly predictable, in situ temperature fluctuations in the global ocean can create discrepancies of up to 3°C between critical temperatures for growth measured in the lab and predicted optima under environmental variability. These discrepancies are on par with the magnitude of predicted ocean warming, highlighting the need to understand the sources and biological consequences of environmental variability.

One of most widely observed patterns among ectotherms is the temperature-size rule (TSR), which describes a decline in body size as temperatures increase. To date, no complete explanation has been found for the why the TSR should be so widely observed, or why smaller body sizes should persist after initial temperature changes, because smaller body size is often
associated with lower fitness. MST takes species’ body masses as a given, leaving unanswered
the question of what drives patterns of body size in the first place. In Chapter 4, I showed that
temperature-dependent body size is governed by a trade-off between resource supply and
demand, and body size decreases with warming as a function of increased metabolic demands.
Importantly, I showed the energetic compensating mechanism underlying body size responses to
warming has fitness implications. Smaller body sizes at warmer temperatures are associated with
higher reproductive output, suggesting that the phenotypic temperature size rule is generated by
physiological and thermodynamic constraints that may have important fitness consequences.

The patterns of variation in abundance, productivity and body size that I documented in Chapters
2 - 4 have important implications for ecosystem services and human well-being. For many of the
world’s 7 billion people, food security is an ecosystem service provided by aquatic ecosystems.
Although biodiversity is known to enhance the provision of ecosystem services in many sectors,
the effect of biodiversity on the nutritional benefits provided by seafood remains unknown. I
have shown that the widespread, repeatable relationship between biodiversity and ecosystem
functioning can yield insight into the benefits that humans derive from aquatic ecosystems. In
Chapter 5, I showed that variation in ecologically important traits such as body size has
important implications for the nutritional benefits of the catch, thus linking the processes that
structure ecosystems with the benefits they provide. This approach has revealed that
understanding what generates and maintains aquatic biodiversity has direct and immediate
consequences for human well-being.
6.2 Revisiting three metabolic currencies: energy, matter and information

At a time of climate change and rapid biodiversity loss, what drives the abundance, distribution and diversity of life is an urgent problem not only for the science of ecology, but also for understanding human well-being. One solution lies in recognizing that all ecological interactions involve exchanges of three fundamental currencies: energy, matter and information. In Chapters 2-4 I highlighted how fluxes of energy and matter at the level of the individual cascade up to shape the dynamics of populations, and ultimately patterns of abundance and persistence. Chapter 5, however, demonstrates that an understanding of the third ecological currency, information, is critical to understanding the relationships between natural systems and human well-being. Predicting the benefits that humans derive from natural systems is not simply about understanding the controls on fluxes of energy and matter, or abundance and productivity. Instead, the delivery of ecosystem services is often tied to particular geographical locations or ecological communities where species’ traits and identity matter (Daily et al. 2009). In the context of seafood-derived human nutrition, not all seafood species are equally nutritious. Total yields are therefore not a good predictor of nutritional value and human nutritional well-being. Seafood species are distinct in their nutritional profiles, and this diversity in nutrition is related in part to taxonomic and trait diversity – demonstrating that the information encoded in species’ genomes and traits is fundamentally related to the benefits that ecosystems provide to people. More generally, information is at the core of the biodiversity-ecosystem function relationships that characterize the connections between biodiversity and human well-being. Taken together, the chapters of this thesis demonstrate that a focus on the three metabolic currencies offers a
productive and efficient way to understand how natural systems change, and the ways in which we rely on them.

6.3 Where to next?

In my experiments, I have chosen to work with highly simplified mesocosms so that I can successfully tease apart the many simplifying assumptions of MST. I have observed that predictions of MST are robust to breaking some of these assumptions, but not others. Future work could incrementally increase the complexity of the systems in which these predictions are tested to continue to test the theory and to refine and expand its predictions.

Another area that is open for further experimentation and theoretical development is the question of how the widely observed activation energies of key metabolic processes evolve. What generates the highly conserved values of 0.32 eV for the activation energy of photosynthesis and 0.65 eV for the activation energy of respiration? Recent work (Padfield et al. 2016, Schaum et al. 2017) has shown the capacity for these activation energies to change over time within a population, but what limits or propels the evolution of temperature dependence remains an open question. Answering this question is critical to achieving a coherent picture of what generates and maintains current and future patterns of abundance, distribution and diversity that are at the core of our discipline.

Finally, I suggest that MST be expanded to account for the fact that all ecological systems are open systems, embedded in a larger spatial context. No understanding of the temperature or mass
dependence of processes at higher levels of organization will be complete without better understanding the effects of transfer of energy, materials and information across ecosystem boundaries. I did not account for spatial processes in my dissertation, not only to restrict the bounds of my questions, but also because, to my knowledge, relevant theory simply does not exist.

To conclude, I have used experiments to make critical tests of several predictions of MST. I have shown that size and temperature scaling of processes operating at the population level can be predicted from organismal level rates, thus filling a key gap in MST. Since population growth rate is a metric of mean fitness in an environment (Roughgarden 1979), linking organismal level processes with population dynamics allows us to make connections between mechanistic metabolic constraints and evolutionary outcomes.
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Appendix A: Chapter 2 supplemental methods and figures

Supplementary methods

Biovolume to biomass conversions

Multiple conversion equations exist to convert phytoplankton cell biovolume to biomass in carbon. The choice of conversion method can influence scaling relationships (Maranon 2007). We used the conversion in Montagnes et al. 1994, because it is appropriate for live chlorophytes. To account for uncertainty in the biovolume to biomass conversion, I also used the conversion proposed by Reynolds (2006), \( \text{ug C} = 0.47(\text{biovolume})^{0.99} \) and Menden-Deuer & Lessard (2000), \( \text{ug C} = 0.358(\text{biovolume})^{1.088} \) and found no significant differences between the methods. I report results using the conversion in Montagnes et al. 1994 in the main text and include results using the other methods in the Appendix (Figures A5 and A6).

Estimation of thermal optimum for exponential growth in Tetraselmis tetrahele

I initiated twenty replicate experimental populations of *T. tetrahele* in 30 mL glass test tubes containing 25 mL of 10uM nitrate ESAW medium (Harrison et al. 1980), at a density of \~ 700 cells/mL under constant temperature conditions at 0°C, 5°C, 10°C, 15°C, 20°C, 24°C, 27°C, 29°C, and 32°C. I sampled each of the twenty replicate populations, with four replicates sampled destructively at each of five time points over the period corresponding to the exponential growth phase at each temperature. I measured population abundances from 250 uL samples using a FlowCAM (flow rate = 0.3 ml/min; FlowCAM VS Series, Fluid Imaging Technologies). I
estimated the temperature dependence of the intrinsic rate of population growth, \( r \), during the exponential growth phase as:

\[
N(t) = N(0)e^{rt}
\]  

(A1),

where \( N(t) \) is the number of individuals at time \( t \) using the \textit{nls.LM} function in the \textit{minpack.LM} package in R (Elzhov et al. 2016). I estimated the temperature dependence of \( r \), \( r(T) \), (Thomas et al. 2012):

\[
r(T) = ae^{bt} \left[ 1 - \left( \frac{T - z}{\frac{w}{2}} \right)^2 \right]
\]  

(A2)

using maximum likelihood estimation with the \textit{mle2} function in the \textit{bbmle} package in R (Bolker 2017). Growth rate, \( r \), is a function of temperature, \( T \), \( a \) and \( b \) are parameters from the Eppley curve (Eppley 1972) that describe maximum observed population growth rates as a function of temperature, \( z \) determines the location of the maximum of the quadratic portion of the function and \( w \) is the range over which the growth rate is positive (i.e. the thermal breadth). We estimated the optimum temperature for exponential growth, \( T_{opt} \), via numerical optimization using the \textit{optim} function in R. The resulting thermal performance curve is shown in Figure A1.
Chapter 2 Supplementary figures

Figure A.1 Thermal performance curve for the exponential growth rate of *T. tetrahele*, with an estimated optimal temperature for growth (\(T_{opt}\)) of 25°C.

Exponential growth rates were estimated at nine temperatures, \(n = 5\) replicate populations per temperature. Points and error bars refer to mean exponential population growth rate and 95% confidence intervals at each temperature; black line refers to Equation A2 fitted to the experimental data.
Figure A.2 Effects of temperature on population abundances over time.

Columns correspond to each of five replicate populations and rows correspond to experimental temperature treatments. Fitted lines are based on parameters estimated from the logistic growth model. Shaded areas represent 95% confidence intervals based on bootstrap resampling and refitting of each dataset (1000 times). $R^2$ values are pseudo-$R^2$ calculated for each replicate population.
Mass normalized carrying capacity (cells mL$^{-1}$ ug C), ($K_NM_i^{3/4}$), decreased with temperature.

Mass normalized carrying capacity (cells mL$^{-1}$ ug C), ($K_NM_i^{3/4}$), decreased with temperature with a slope of -0.22 eV (95% CI: -0.25, -0.18; n = 5 replicates per temperature). Solid line is the linear model fit to data with shaded area representing 95% confidence intervals. Open circles are from experimental populations grown above the optimum temperature for growth ($T_{opt}$, 25°C), demonstrating that carrying capacity declined much more rapidly past $T_{opt}$. Dotted line corresponds to the predicted carrying capacity from Equation 7 with a temperature-independent body mass ($\beta = 0$). Thick dashed line corresponds to predicted carrying capacity, with a temperature-dependent body mass (Equation 7, $\beta = 1.92\%$). For ease of interpretation, a secondary horizontal axis (upper axis) shows temperature in °C.
Figure A.4 Cell size decreased with increasing temperature.

Cell size decreased with increasing temperature across a temperature gradient of 5°C – 25°C, shown at five time points over the course of the experiment. Shaded bands refer to 95% confidence intervals around OLS regression lines, n = 5 replicate populations per temperature.
A) Reynolds

**Photosynthesis**

Temperature (°C)

\[ E_G = 0.33 \text{ (95\% CI: 0.20, 0.45)} \]

\[ y = -0.33x + 0.57 \quad R^2 = 0.21 \quad n = 18 \]

**Respiration**

Temperature (°C)

\[ E_R = 0.52 \text{ (95\% CI: 0.39, 0.66)} \]

\[ y = -0.52x + 7.77 \quad R^2 = 0.38 \quad n = 18 \]

B) Menden-Deuer

Temperature (°C)

\[ E_G = 0.33 \text{ (95\% CI: 0.20, 0.46)} \]

\[ y = -0.33x - 0.90 \quad R^2 = 0.22 \quad n = 18 \]

\[ E_R = 0.52 \text{ (95\% CI: 0.39, 0.66)} \]

\[ y = -0.52x + 6.30 \quad R^2 = 0.38 \quad n = 18 \]

C) Montagnes

Temperature (°C)

\[ E_G = 0.33 \text{ (95\% CI: 0.20, 0.45)} \]

\[ y = -0.33x - 7.10 \quad R^2 = 0.22 \quad n = 18 \]

\[ E_R = 0.52 \text{ (95\% CI: 0.39, 0.66)} \]

\[ y = -0.52x + 0.035 \quad R^2 = 0.39 \quad n = 18 \]
Figure A.5 Mass normalized metabolic rates of *T. tetrahele* increased with temperature.

Mass-normalized metabolic rates ($B_i M_i^{-3/4}$) of *T. tetrahele* increased with temperature for gross photosynthesis and for respiration. Points are shown at medium opacity to indicate overlap, shaded bands refer to 95% confidence intervals from OLS regression. Mass normalized metabolic rates were converted from biovolume to biomass with different conversion equations; (A) Reynolds 2006; (B) Menden-Deuer and Lessard 2000; (C) Montagnes et al. 1994. For ease of interpretation, a secondary horizontal axis (upper axis) shows temperature in °C.
Carrying capacity estimated from time series of population biovolume converted to population biomass with three different biovolume to biomass conversion equations: Menden-Deuer and Lessard (2000) (A), Montagnes et al. 1994 (B) and Reynolds (2006) (C). Shaded bands refer to 95% confidence intervals around OLS regression lines. For ease of interpretation, a secondary horizontal axis (upper axis) shows temperature in °C.
Figure A.7 Ratio of mass-normalized gross photosynthesis to respiration.

Ratio of mass-normalized gross photosynthesis to respiration declined over a temperature gradient from 8°C to 24°C (slope = -0.20 eV, 95% CI: -0.31, -0.03, Adjusted $R^2 = 0.10$, n = 18 per temperature). Shaded bands refer to 95% confidence intervals around OLS regression line. For ease of interpretation, a secondary horizontal axis (upper axis) shows temperature in °C.
Figure A.8 Estimates of carrying capacity, $K_N$ over a temperature gradient.

Points and error bars represent means and 95% confidence intervals from bootstrap resampling the time series and refitting the logistic model 1000 times.
Appendix B: Chapter 3 supplemental methods and figures

Estimating the temperature dependence of population growth rates

I estimated the temperature dependence of population growth in two ways: 1) a ‘direct’ approach in which I estimated the temperature dependence of population growth directly from time series of population abundance for populations grown at 9 temperatures spanning a temperature gradient (Chapter 3, Equations 2 and 3) and 2) an ‘indirect’ approach in which we first estimated population growth rates at each experimental temperature separately, and then fit a thermal performance curve (Equation 3) to the estimated growth rates at each temperature. These two approaches are complementary and have different strengths (Palamara et al. 2014): while the ‘direct’ approach enables more accurate estimation of the parameters of the functional form of temperature dependence, the ‘indirect’ approach may yield a clearer picture of the shape of the functional form of temperature dependence. I describe the methods and results from the ‘direct’ approach in the main text and the ‘indirect’ approach in the supplement (Figures B.2, B.6), and display estimates from the ‘direct’ approach in Figure 2.

Statistical estimation of performance curves using the ‘indirect’ method

I estimated the intrinsic rate of population growth, \( r \), during the exponential growth phase as:

\[
N(t) = N(0)e^{rt}
\]  

where \( N(t) \) is the number of individuals at time \( t \), using non-linear least squares regression with the \textit{nls} function in R (R Core Team 2017).
For constant and variable conditions, I fit a thermal performance curve for growth rate (Thomas et al. 2012),

$$r(T) = ae^{bT} \left[ 1 - \left( \frac{T - z}{w} \right)^2 \right]$$  \hspace{1cm} (S2)

where growth rate, $r$, is a function of temperature, $T$; $w$ is the range over which the growth rate is positive (i.e. the thermal breadth); $z$ sets the location of the maximum of the quadratic portion of the function; $a$ and $b$ are parameters from the Eppley curve (Eppley 1972) that describe maximum observed growth rates as function of temperature. I estimated these parameter values using maximum likelihood estimation with the mle2 function in the bblme package (Bolker 2017).

To generate estimates of uncertainty in critical temperatures of the TPC (e.g. $T_{opt}$), I determined confidence intervals around fitted thermal performance curves using parametric bootstrapping, following the approach outlined in Equation S.3 in (Thomas et al. 2012). I then used the TPC fitted under constant conditions and its upper and lower confidence intervals to make predictions under variable conditions. For the thermal performance curve fitted to the nine experimental temperatures under constant conditions, I simulated nine new performance values drawn from a normal distribution such that the mean of the distribution corresponded to the value of Equation 3 at each of the original experiment temperatures, given the estimated parameters and the
standard deviation = \sigma, where \sigma was obtained by accounting for uncertainty in the estimation of the original maximum likelihood standard deviation, \tilde{\sigma}, using:

\[ \tilde{\sigma}^2 = \sigma^2 \frac{n-1}{\chi} \]  

(S3)

where \( n \) is the number of data points (\( n = 9 \)), and \( \chi \) is a random number drawn from the chi-squared distribution having (\( n – 1 \)) degrees of freedom (Gelman and Hill 2007). I then fit Equation S2 to these nine simulated performance estimates using maximum likelihood estimation and repeated this process 5000 times to generate 5000 new thermal performance curves.

Using scale transition theory (Taylor approximation) to predict population growth rates in thermally variable environments

When empirical time series of body temperatures are available, then predictions for performance in fluctuating environments can be made using Equation 1 in Chapter 3. However, an alternative approach to estimating expected performance under variable conditions when only the mean and variance of the temperature distribution are available is to approximate expected performance under variable conditions using a Taylor approximation of the TPC, an approach incorporated into scale transition theory (Chesson et al. 2005). Scale transition theory builds on Jensen’s inequality, and makes predictions for how non-linear dynamics change over spatial and temporal scales. Scale transition theory was developed for metacommunity models and allows for quantitative predictions for how variation at one scale emerges at another when mediated by a
nonlinear relationship such as a TPC. Here, I use a Taylor approximation approach from scale transition theory in the context of relating estimated performance over time-averaged scales to performance at short time scales generated under static conditions. If $T$ is a random variable with mean $\bar{T}$ and variance $\sigma^2_T$ then we can approximate $E(r)$ using a Taylor expansion of $r = f(T)$ around $\bar{T}$ (Estay et al. 2014). The second order Taylor expansion of $f(T)$ is:

$$r \approx f(\bar{T}) + (T - \bar{T}) f'(\bar{T}) + \frac{1}{2} (T - \bar{T})^2 f''(\bar{T}) \quad (S4)$$

Taking the mathematical expectation of both sides of Equation S4, the expected growth rate under variable conditions can be approximated by:

$$E(r) \approx f(\bar{T}) + \frac{1}{2} f''(\bar{T}) \sigma^2_T \quad (S5)$$

since $E(T - \bar{T}) = 0$ and $E(T - \bar{T})^2 = \sigma^2_T$ is the variance of temperatures over time. Equation S5 has been incorporated into scale transition theory (Chesson et al. 2005, Estay et al. 2014, Dowd et al. 2015), which builds on Jensen’s inequality, and makes predictions for how non-linear dynamics change over spatial and temporal scales. Equation S5 will be most accurate when the higher moments (variance, skew and kurtosis) of the distribution of temperatures over time are small and the higher order terms of the Taylor expansion are small, such that $f(T)$ is not highly non-linear around temperature $T$. By quantifying the curvature at a given point on a thermal performance curve, Equation S5 provides a way to mathematically predict the strength and direction of effects of temperature fluctuations around that temperature on performance, even when empirical time series of temperature are not available. I present the results from the scale transition theory method in the supplement to enable comparisons between predictions using non-linear averaging over the entire temporal temperature distribution in the main text vs. just the mean and variance using the scale transition theory approach (Figures B.3, B.4, B.5).
Quality control criteria for the global phytoplankton dataset

I restricted my analysis of the global phytoplankton dataset (Thomas et al. 2016) to marine species that met the following conditions: 1) experimental population growth rate estimates were available for at least five temperatures (n = 89 phytoplankton strains), 2) estimated $T_{\text{max}}$ and $T_{\text{min}}$ were within 5°C of the highest and lowest measurement temperatures respectively, 3) growth rates at two or more temperatures above and below $T_{\text{opt}}$ were reported, and 5) fitted curves had an $R^2$ of at least 0.85, to ensure the estimated parameters describing the shape of the curve accurately captured the curvature of the TPCs. In addition to these curve shape criteria, all the curves in the dataset met the quality control criteria outlined in (Thomas et al. 2016) (namely that the experimental populations were grown with adequate light and nutrient supply, and non-stressful pH and salinities).
Figure B.1 Growth-irradiance curve for *T. tetrahele* grown at 27°C.

Growth-irradiance curve for *T. tetrahele* grown at 27°C, a temperature close to *T* \(_{\text{opt}}\) in this population, fit with the Eilers and Peeters dynamic model of photoinhibition of photosynthesis (Eilers and Peeters 1988): 

\[
\mu(I) = \frac{\mu_{\text{max}} I}{\alpha} \left( 1 - \frac{\mu_{\text{max}} I}{\alpha} \right) \left( 1 - 2 \frac{\mu_{\text{max}} I}{\alpha} \right) \frac{\mu_{\text{max}} I}{\alpha},
\]

where \(\mu\) is the growth rate (day\(^{-1}\)) and a function of photon flux density \((I, \text{\(\mu\)mol photons m}^2\text{s}^{-1})\), \(\mu_{\text{max}}\) is the maximum growth rate at \(I_{\text{opt}}\), and \(\alpha\) is the initial slope of the curve.
Figure B.2 Thermal performance curves for *T. tetrahele* populations growing under constant and variable temperature conditions; growth rates estimated by the ‘indirect’ approach.
A) Exponential growth rates under constant temperature conditions; blue line is the fitted thermal performance curve and blue shading corresponds to 95% CI generated from parametric bootstrapping (Appendix B). The dashed curve represents predicted growth rate under thermally variable conditions based on nonlinear averaging (Equation 1). Points and error bars are observed mean growth rates and 95% CI. B) Exponential growth rates under thermally fluctuating conditions (±5°C); dashed curve is predicted based on panel A and Equation 1, green line is the fitted thermal performance curve under fluctuating conditions, green shading corresponds to 95% CI generated from parametric bootstrapping. Green points and error bars (95% CI) are observed mean growth rates in the fluctuating temperature regime.
Figure B.3 Comparison of predictions of population growth rates in a variable environment using nonlinear averaging (Equation 1, black dashed lines) and scale transition theory (Equation S5, grey dashed lines).

Predictions are shown for growth rates in temperature fluctuations of 5°C above and below each mean temperature. The constant temperature TPC (blue line, and blue shading for 95% CI generated by non-parametric bootstrapping) was estimated via the ‘direct approach’ outlined in Chapter 3 (Equations 2 and 3) from experimental populations of *Tetraselmis tetrahele* grown over a temperature gradient.
Figure B.4 Thermal performance curves (green curves) and $T_{opt}$ (green vertical lines) overlaid on frequency histograms of daily temperatures from 1981 – 2011 at the isolation location for each phytoplankton strain.

Grey vertical lines show the mean annual temperature at each isolation location. Mean annual temperatures at isolation locations are typically lower than $T_{opt}$. Orange curves represent the predicted population growth rate using nonlinear averaging given the distribution of daily temperatures (Equation 1), black dashed lines represent predicted growth rates made with scale transition theory using the mean and variance of the temperature distribution (Equation S5). Black dashed lines (scale transition theory predictions) are plotted on top of orange lines (nonlinear averaging predictions), and in many cases the curves are similar enough to appear as one line in this plot. Numbers at the top of each panel correspond to each isolate’s unique ID, ‘isolate code’, in (Thomas et al. 2016).
Figure B.5 Comparison of *in situ* growth rates estimates, $\bar{r}$, estimated with scale transition theory (Taylor series approximation) and non-linear averaging.

Skewness of the TPC is shown in the color scale. 1:1 line is shown in black.
Comparison of $r_{max}$, $T_{opt}$ and $T_{max}$ (blue and green circles) for experimental populations of *Tetraselmis tetrahele* in constant and variable thermal environments estimated via the ‘direct’ approach (i.e. when the temperature dependence of population growth rate is estimated directly from time series of population abundance over a temperature gradient) vs the ‘indirect’ approach (i.e. when growth rates are estimated at each temperature separately, and then the thermal performance curve is fit to the series of temperature-dependent growth rate estimates). Green
triangles refer to predictions for $r_{\text{max}}$, $T_{\text{opt}}$, and $T_{\text{max}}$ generated from nonlinear averaging (Equation 1) applied over the constant-conditions TPC.
Figure B.7 Time series of population abundance in experimental populations of *Tetraselmis tetrahele* grown in constant and variable thermal regimes

Time series of population abundance in experimental populations of *Tetraselmis tetrahele* grown in constant and variable thermal regimes. Lines show the predicted population growth rates ($r$) over the temperature gradient from the non-linear regression model (Equation 2 and 3 in Chapter 3) fitted via the ‘direct’ approach. Only the exponential portion of the population growth trajectories are shown.
Appendix C: Chapter 5 supplemental figures and tables

Figure C.1 The effect of biodiversity on nutritional diversity ($N_D$) depends on the DRI threshold.

A) Number of distinct DRI targets reached per 100g edible portion increases with species richness. B) The effect of biodiversity on nutritional diversity ($N_D$) is strongest at a threshold of approximately 28% of DRI.
Figure C.2 Biodiversity enhances nutritional benefits in terms of two metrics of nutritional benefit: nutritional efficiency ($N_E$) (A) and nutritional diversity ($N_D$) (B).

Each point corresponds to the $b$ parameter estimate from $y = aS^b$, where $y =$ nutritional benefit ($N_E$ or $N_D$) and each point corresponds to one of fourteen local indigenous diets and the global diet (GL; standardized to 40 species). Points are means ± 95% CI from non-parametric bootstrapping of the fit of Equations 1 (panel A) and 2 (panel B) to randomly assembled diets. Names of regions for each local diet are represented in two-letter abbreviations which are listed in Table C7.
Among finfish species, nutrient concentration varies by body part in the edible portion. Fish species that are eaten whole, or whose edible portions include organs such as skin, liver or bones have higher nutrient concentrations than those whose edible portions are restricted to muscle tissue. Points are means ± standard error.
Nutrient concentrations are typically weakly correlated or uncorrelated among species. Shading corresponds to correlation coefficients, squares covered with an ‘X’ are significantly correlated; \( p < 0.05 \).

Figure C.4 Correlations among nutrient concentrations among species.
Figure C.5 Observed vs Expected functional diversity (\(FD\)) and functional evenness (\(FEve\)) in local and global diets.
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Table C.1 Percentage of species that reach 10% of DRI, and total number of species (n) grouped by taxonomic group.
The variables included in each model are shown with the symbol •. Models are ranked in order of increasing $\Delta \text{aic}$. Akaike weights ($w$) indicate the relative likelihood of a model, given the particular set of best models being considered (Burnham and Anderson 2002). Model-averaged regression coefficients ($\beta$) are averages of $\beta$ across all models with $\Delta \text{aic} < 2$, weighted by each model's Akaike weight $w$. Calculations for $\beta$ include $\beta = 0$ when variables are not in a given model. $\beta$ whose 95% confidence intervals do not encompass zero are given in bold.
## Table C.3 Results of model averaging and model selection for calcium.

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<td>0.74</td>
<td>0.74</td>
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</tr>
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<td>$\delta$aic</td>
<td>0.00</td>
<td>2.23</td>
<td>5.32</td>
<td>8.83</td>
<td>11.07</td>
<td>13.20</td>
</tr>
<tr>
<td>$w$</td>
<td>0.71</td>
<td>0.23</td>
<td>0.050</td>
<td>0.0086</td>
<td>0.0028</td>
<td>0.00096</td>
</tr>
</tbody>
</table>

The variables included in each model are shown with the symbol •. Models are ranked in order of increasing $\delta$aic. Akaike weights ($w$) indicate the relative likelihood of a model, given the particular set of best models being considered (Burnham and Anderson 2002). Model-averaged regression coefficients ($\beta$) are averages of $\beta$ across all models with $\delta$aic < 2, weighted by each model's Akaike weight $w$. Calculations for $\beta$ include $\beta = 0$ when variables are not in a given model. $\beta$ whose 95% confidence intervals do not encompass zero are given in bold.
Table C.4 Results of model averaging and model selection for zinc.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Model rank</th>
<th>Model average</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>ln(body size)</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Trophic position</td>
<td></td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Herbivore grazer</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Omnivore</td>
<td></td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Latitude</td>
<td></td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Feeding mode grazer</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Feeding mode active predator</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Feeding mode filter feeder</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Feeding mode variable</td>
<td></td>
<td>•</td>
<td>•</td>
</tr>
</tbody>
</table>

Marginal $R^2$          | 0.15       | 0.14          | 0.16    | 0.17    | 0.16    | 0.014   |
Conditional $R^2$        | 0.70       | 0.72          | 0.79    | 0.68    | 0.69    | 0.74    |
$\delta$aic              | 0.00       | 0.62          | 0.19    | 2.63    | 3.35    | 5.20    |
$w$                      | 0.355      | 0.261         | 0.196   | 0.095   | 0.067   | 0.026   |

The variables included in each model are shown with the symbol •. Models are ranked in order of increasing $\delta$aic. Akaike weights ($w$) indicate the relative likelihood of a model, given the particular set of best models being considered (Burnham and Anderson 2002). Model-averaged regression coefficients ($\bar{\beta}$) are averages of $\beta$ across all models with $\delta$aic < 2, weighted by each model's Akaike weight $w$. Calculations for $\beta$ include $\beta = 0$ when variables are not in a given model. $\beta$ whose 95% confidence intervals do not encompass zero are given in bold.
The variables included in each model are shown with the symbol •. Models are ranked in order of increasing $\delta$AIC. Akaike weights ($w$) indicate the relative likelihood of a model, given the particular set of best models being considered (Burnham and Anderson 2002). Model-averaged regression coefficients ($\beta$) are averages of $\beta$ across all models with $\delta$AIC < 2, weighted by each model's Akaike weight $w$. Calculations for $\beta$ include $\beta = 0$ when variables are not in a given model. $\beta$ whose 95% confidence intervals do not encompass zero are given in bold.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Model rank</th>
<th>Model average</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>ln(body size)</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>Trophic position</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>Herbivore grazer</td>
<td>•</td>
<td>•</td>
<td></td>
<td></td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>Omnivore</td>
<td>•</td>
<td>•</td>
<td></td>
<td></td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>Latitude</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>$\beta$</td>
<td>-0.21</td>
<td>-0.40 to -0.015</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>-0.57 to 0.057</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marginal $R^2$</td>
<td>0.015</td>
<td>0.020</td>
<td>0.022</td>
<td>0.020</td>
<td>0.021</td>
<td>0.022</td>
</tr>
<tr>
<td>Conditional $R^2$</td>
<td>0.82</td>
<td>0.82</td>
<td>0.82</td>
<td>0.82</td>
<td>0.82</td>
<td>0.82</td>
</tr>
<tr>
<td>$\delta$AIC</td>
<td>0.00</td>
<td>1.02</td>
<td>6.32</td>
<td>6.82</td>
<td>9.48</td>
<td>10.24</td>
</tr>
<tr>
<td>$w$</td>
<td>0.591</td>
<td>0.356</td>
<td>0.025</td>
<td>0.020</td>
<td>0.005</td>
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Table C.5 Results of model averaging and model selection for EPA.
Table C.6 Results of model averaging and model selection for DHA.

<table>
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<tr>
<th>Trait</th>
<th>Model rank</th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th>Model average</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>$\beta$</td>
</tr>
<tr>
<td>ln(body size)</td>
<td>$\bullet$</td>
<td>$\bullet$</td>
<td>$\bullet$</td>
<td>$\bullet$</td>
<td></td>
<td></td>
<td>$-0.21$</td>
</tr>
<tr>
<td>Trophic position</td>
<td>$\bullet$</td>
<td>$\bullet$</td>
<td></td>
<td></td>
<td>$\bullet$</td>
<td>$\bullet$</td>
<td>$0.10$</td>
</tr>
<tr>
<td>Herbivore grazer</td>
<td>$\bullet$</td>
<td></td>
<td></td>
<td>$\bullet$</td>
<td>$\bullet$</td>
<td>$\bullet$</td>
<td>$-0.27$</td>
</tr>
<tr>
<td>Omnivore</td>
<td></td>
<td></td>
<td>$\bullet$</td>
<td>$\bullet$</td>
<td>$\bullet$</td>
<td>$\bullet$</td>
<td>$-0.31$</td>
</tr>
<tr>
<td>Latitude</td>
<td>$\bullet$</td>
<td></td>
<td></td>
<td></td>
<td>$\bullet$</td>
<td>$\bullet$</td>
<td>$0.0071$</td>
</tr>
<tr>
<td>Feeding mode grazer</td>
<td>$\bullet$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$0.38$</td>
</tr>
<tr>
<td>Feeding mode active predator</td>
<td>$\bullet$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$0.072$</td>
</tr>
<tr>
<td>Feeding mode filter feeder</td>
<td>$\bullet$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$0.70$</td>
</tr>
<tr>
<td>Feeding mode variable</td>
<td>$\bullet$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$-0.18$</td>
</tr>
<tr>
<td>Marginal $R^2$</td>
<td>0.022</td>
<td>0.010</td>
<td>0.024</td>
<td>0.016</td>
<td>0.018</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td>Conditional $R^2$</td>
<td>0.81</td>
<td>0.80</td>
<td>0.81</td>
<td>0.80</td>
<td>0.81</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>$\delta_{aic}$</td>
<td>0.00</td>
<td>0.45</td>
<td>1.62</td>
<td>1.82</td>
<td>2.26</td>
<td>5.35</td>
<td></td>
</tr>
<tr>
<td>$w$</td>
<td>0.329</td>
<td>0.263</td>
<td>0.146</td>
<td>0.133</td>
<td>0.106</td>
<td>0.023</td>
<td></td>
</tr>
</tbody>
</table>

The variables included in each model are shown with the symbol $\bullet$. Models are ranked in order of increasing $\delta_{aic}$. Akaike weights ($w$) indicate the relative likelihood of a model, given the particular set of best models being considered (Burnham and Anderson 2002). Model-averaged regression coefficients ($\beta$) are averages of $\beta$ across all models with $\delta_{aic} < 2$, weighted by each model's Akaike weight $w$. Calculations for $\beta$ include $\beta = 0$ when variables are not in a given model. $\beta$ whose 95% confidence intervals do not encompass zero are given in bold.
<table>
<thead>
<tr>
<th>Culture</th>
<th>Abbreviation</th>
<th>Region</th>
<th>Location</th>
<th>Species in diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abenaki</td>
<td>AB</td>
<td>Northeast</td>
<td>Quebec; Maine</td>
<td>29</td>
</tr>
<tr>
<td>Bella Coola</td>
<td>BC</td>
<td>Northwest</td>
<td>British Columbia</td>
<td>40</td>
</tr>
<tr>
<td>Central Salish</td>
<td>CS</td>
<td>Northwest</td>
<td>British Columbia; Washington</td>
<td>42</td>
</tr>
<tr>
<td>Cree</td>
<td>CR</td>
<td>Subarctic</td>
<td>Labrador; Quebec; Ontario; Manitoba; Saskatchewan; Alberta</td>
<td>27</td>
</tr>
<tr>
<td>Haida</td>
<td>HA</td>
<td>Northwest</td>
<td>British Columbia; Alaska</td>
<td>36</td>
</tr>
<tr>
<td>Inuit-Inupiaq</td>
<td>II</td>
<td>Arctic</td>
<td>Alaska; Northwest Territories; Nunavut; Nunavik; Quebec; Labrador</td>
<td>52</td>
</tr>
<tr>
<td>Kwakiutl</td>
<td>KW</td>
<td>Northwest</td>
<td>British Columbia</td>
<td>40</td>
</tr>
<tr>
<td>Micmac</td>
<td>MI</td>
<td>Northeast</td>
<td>Nova Scotia; New Brunswick; Quebec; Newfoundland</td>
<td>57</td>
</tr>
<tr>
<td>Montagnais-Naskapi</td>
<td>MN</td>
<td>Subarctic</td>
<td>Labrador; Quebec</td>
<td>25</td>
</tr>
<tr>
<td>Nootkan</td>
<td>NO</td>
<td>Northwest</td>
<td>British Columbia; Washington</td>
<td>49</td>
</tr>
<tr>
<td>Tlingit</td>
<td>TL</td>
<td>Northwest</td>
<td>British Columbia; Yukon; Alaska</td>
<td>51</td>
</tr>
<tr>
<td>Tsimshian</td>
<td>TS</td>
<td>Northwest</td>
<td>British Columbia; Alaska</td>
<td>41</td>
</tr>
<tr>
<td>Wampanoag</td>
<td>WA</td>
<td>Northeast</td>
<td>Massachusetts</td>
<td>35</td>
</tr>
<tr>
<td>Yupik</td>
<td>YU</td>
<td>Arctic</td>
<td>Alaska</td>
<td>38</td>
</tr>
</tbody>
</table>

Table C.7 Names and locations of North American indigenous cultures used in the local scale nutritional diversity analysis.
**Table C.8** Top fourteen of 41 most commonly consumed species as per FAO production volumes in the nutrient dataset. Data source: (Food and Agriculture Organization 2013).

<table>
<thead>
<tr>
<th>Genus</th>
<th>species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theragra</td>
<td>chalcogramma</td>
</tr>
<tr>
<td>Gadus</td>
<td>morhua</td>
</tr>
<tr>
<td>Gadus</td>
<td>macrocephalus</td>
</tr>
<tr>
<td>Tenualosa</td>
<td>ilisha</td>
</tr>
<tr>
<td>Rastrelliger</td>
<td>kanagurta</td>
</tr>
<tr>
<td>Merluccius</td>
<td>productus</td>
</tr>
<tr>
<td>Oncorhynchus</td>
<td>gorbuscha</td>
</tr>
<tr>
<td>Pollachius</td>
<td>virens</td>
</tr>
<tr>
<td>Melanogrammus</td>
<td>aeglefinus</td>
</tr>
<tr>
<td>Thunnus</td>
<td>alalunga</td>
</tr>
<tr>
<td>Oreochromis</td>
<td>niloticus</td>
</tr>
<tr>
<td>Penaeus</td>
<td>monodon</td>
</tr>
<tr>
<td>Portunus</td>
<td>pelagicus</td>
</tr>
<tr>
<td>Trachurus</td>
<td>trachurus</td>
</tr>
<tr>
<td>Categorical predictors</td>
<td>Levels</td>
</tr>
<tr>
<td>------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Feeding mode</td>
<td>browsing on substrate&lt;br&gt;feeding on the prey of a host (commensal)&lt;br&gt;filtering plankton&lt;br&gt;grazing on aquatic plants&lt;br&gt;hunting macrofauna (predator)&lt;br&gt;other&lt;br&gt;selective plankton feeding&lt;br&gt;sucking food-containing material&lt;br&gt;variable</td>
</tr>
<tr>
<td>Diet breadth</td>
<td>mainly animals&lt;br&gt;mainly plants/detritus&lt;br&gt;plants/detritus+animals</td>
</tr>
<tr>
<td>Continuous predictors</td>
<td>Min</td>
</tr>
<tr>
<td>ln(body size)</td>
<td>1.95</td>
</tr>
<tr>
<td>absolute latitude</td>
<td>0.03</td>
</tr>
<tr>
<td>fractional trophic position</td>
<td>2</td>
</tr>
</tbody>
</table>

Table C.9 Predictors used in linear mixed effects models.