Global change effects on zooplankton body size: a range of experimental approaches

Dissertation

zur Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultät

der Christian-Albrechts-Universität zu Kiel

vorgelegt von

Jessica Garzke

Kiel, 2014

Referent: Prof. Dr. Ulrich Sommer

Koreferent: Prof. Dr. Stefanie M.H. Ismar

Tag der mündlichen Prüfung: 15.10.2014

Zum Druck genehmigt: 15.10.2014

Table of contents

SUMMARY

It is a major challenge to understand the impacts of recent climate change on zooplankton communities. The impacts of global warming are manifold and multiple factors, which drive ecological changes in zooplankton communities have to be understood. Increasing sea surface temperature is likely to alter zooplankton phenology and community structure. Recent studies on the global scale showed a decline in size and productivity of zooplankton, which was related to climate change. Reorganization of zooplankton community with warming can change community interactions and energy flow through whole aquatic food webs.

The aim of this thesis was to examine the effects of temperature, phosphorus limitation and acidification on copepods and disentangle direct and indirect effects of warming on zooplankton and how the observed changes can alter the metabolic fluxes in food webs. In the first chapter, I tested the effect of three temperatures on copepod communities in a mesocosm experiment. The second chapter presents results of a monoculture experiment with the copepod species *Acartia tonsa*, where phosphorus concentration in food algae and temperature effects were combined. In my third experiment, a second mesocosm study, I show effects of the combined factors temperature and ocean acidification, to understand single and interactive effects on the copepod community. With respect to trophic chain length, I demonstrate in chapter 4, that total and mass-specific ecosystem primary production and respiration are differently temperature sensitive, and that bloom dynamics and non-bloom dynamics act differently on ecosystem oxygen fluxes.

In the first chapter of this thesis, I describe the results of a performed mesocosm experiment, which allowed me to identify copepod responses to temperature. Body size of adult copepods and of all *Acartia* sp. developmental stages was smaller at higher temperatures. Total zooplankton, nauplii and adult copepod abundance was lower at higher temperatures. Additionally, a stage shift from older, at lower temperatures, to younger developmental stages occurred at higher temperatures.

My experimental work, presented in chapter 2, focused on the identification of drivers that lead to smaller body sizes. A monoculture experiment was performed with the copepod species *Acartia tonsa* grown under five temperature steps (10 – 20 °C) and were fed with *Rhodomonas salina*, which had three different carbon-to-phosphorus ratios (phosphorus replete, limited, and pulsed). An increase in temperature significantly reduced the individual body size of the herbivore consumer, whereas phosphorus limitation had no influence. Phosphorus limitation counteracts the temperature effect by decreasing developmental rates. I concluded that phosphorus limitation and increasing temperature might accelerate growth rates because temperature has a stronger effect on *Acartia tonsa* than phosphorus limitation.

In chapter 3, I show that warming has a stronger impact on copepod body size, abundance, biomass, and fatty acid composition, whereas acidification did not show a significant effect on copepods. I could identify trends of acidification effects on copepods, with the result that copepods are more positively affected by the fertilizing effect on phytoplankton biomass, as a proxy for food biomass. It seems that copepods were able to partially compensate the negative temperature effects by higher food uptake. Fatty acid composition was significantly affected by warming. Total fatty acid amount did not change with temperature or acidification,

but ratios of single essentially polyunsaturated fatty acids to total fatty acid content changed significantly. I concluded that acidification has the potential to dampen temperature effects on copepod body size, abundance, and biomass by a higher availability of food sources. Copepod populations might be more affected by warming than by ocean acidification alone.

My experimental work, presented in chapter 4, focused on a multi-generational mesocosm study assessing how warming affects ecosystem metabolic rates of net primary production and respiration. A decrease of phytoplankton biomass, zooplankton body size and abundance increase the temperature driven increase of metabolic rates. The results indicate that future warmer aquatic ecosystems are more affected at higher trophic levels, because of changing food web structure to lower phytoplankton biomass, zooplankton abundance with smaller sized individuals. I suggest that biomass changes have to be included into ecosystem flux analyses.

To summarize the results of my experimental studies, I developed a schematic of temperature impacts on biotic interactions in aquatic plankton communities. In this schematic temperature can directly act on copepod metabolic rates, which directly lead to smaller sized individuals and lower abundances, and consequently lower biomass of copepods. Higher metabolic rates lead to higher energy demands and increase the grazing rates on phytoplankton. The increased grazing rates indirectly affect phytoplankton communities.

My work highlights the importance of complex community studies including the interactions with different trophic levels for understanding ecological processes in aquatic ecosystems and their responses to predicted global change scenarios. Testing multiple factors and also further effects on the organism's physiological level, which might result in changes of whole food web efficiency, might achieve the elucidation of the complexity of responses.

ZUSAMMENFASSUNG

Es stellt eine große Herausforderung dar, die Auswirkungen der jüngsten Klimaveränderungen auf Zooplanktongemeinschaften zu verstehen. Die Einflüsse der globalen Erwärmung sind mannigfaltig und viele Faktoren müssen verstanden werden, die die ökologischen Veränderungen in Zooplanktongemeinschaften antreiben. Ansteigende Temperaturen der Meeresoberfläche können wahrscheinlich Phänologie und Gemeinschaftsstruktur verändern. Jüngste Studien zeigten weltweite Abnahmen von Körpergrößen und Produktivität des Zooplanktons, die mit den Klimaveränderungen in Verbindung gebracht werden. Die Umstrukturierung der Zooplanktongemeinschaft kann zu Veränderungen von Interaktionen innerhalb der Planktongemeinschaft führen, sowie deren Energieflüsse durch das gesamte Nahrungsnetz ändern.

Das Ziel dieser Arbeit war, den Einfluss von Temperatur, Phosphat-Limitierung und Ozeanversauerung auf Kopepoden zu untersuchen und die direkten und indirekten Effekte auf Zooplankton voneinander zu trennen, sowie die Auswirkungen der sich veränderten metabolischen Flüsse in Nahrungsnetzen einzuschätzen. Im ersten Kapitel untersuchte ich die Auswirkungen von drei verschiedenen Temperaturen auf Kopepodengemeinschaften mit Hilfe eines Mesokosmen-Experimentes. Das zweite Kapitel präsentiert Ergebnisse eines Monokulturen-Experimentes, bei dem verschiedene Phosphatkonzentration in Futteralgen und der Effekt verschiedener Temperaturen kombiniert wurde. In meinem dritten Experiment, einer Mesokosmos-Studie, zeige ich die Auswirkungen von Temperatur und Ozeanversauerung, um die Effekte von beiden einzelnen sowie gekreuzten Faktoren auf die Kopepodengemeinschaft zu verstehen. In Hinblick auf verschieden Längen von Nahrungsketten, konnte ich in Kapitel 4 zeigen, dass es Unterschiede zwischen gesamter und massen-spezifischer Ökosystem Netto-Primär-Produktion sowie Respiration gibt. Es zeigte sich, dass Temperatur unterschiedlich auf den Sauerstofffluss zwischen dynamischen (Blüten) und stabilen (nach einer Blüte) Perioden wirkt.

In Kapitel eins dieser Thesis beschreibe ich die Ergebnisse eines durchgeführten Mesokosmen Experimentes. Dieses Experiment ermöglichte es mir, die Reaktionen von Kopepoden auf Temperaturveränderungen zu identifizieren. Die Körpergröße von geschlechtsreifen Kopepoden und von Individuen aller Entwicklungsstadien der Gattung *Acartia* sp. hatten kürzere Prosomenlängen bei höheren Temperaturen. Abundanzen der gesamten Zooplankton-, Nauplien und geschlechtsreifen Kopepoden waren niedriger bei höheren Temperaturen. Zusätzlich erfolgte eine Veränderung der durchschnittlichen Entwicklungsstadien Indices von älteren Tieren bei niedrigeren Temperaturen zu jüngeren bei höheren Temperaturen.

Meine experimentelle Arbeit in Kapitel 2, befasst sich mit der Identifizierung von Treibern, die zu kleiner Körpergröße führen. Ein Monokulturen-Experiment wurde mit der Kopepodenart *Acartia tonsa* durchgeführt, die bei fünf unterschiedlichen Temperaturen wuchsen (10 - 20 °C) und mit der Alge *Rhodomonas salina* gefüttert wurden (drei verschiedene Kohlenstoff-zu-Phosphat Verhältnisse: Phosphat gesättigt, limitiert und gepulst). Zunehmende Temperaturen verringerten signifikant die individuelle Körpergröße von *Acartia tonsa*, wogegen Phosphat-Limitation keinen Einfluss hatte. Phosphat-Limitation wirkt entgegen dem Effekt von höheren Temperaturen auf die Entwicklungsraten.

Ich schließe aus diesen Ergebnissen, dass Phosphat-Limitation und steigende Temperaturen die Wachstumsraten erhöhen, weil der Temperatureffekt auf *Acartia tonsa* stärker wirkt als der von Phosphat-Limitation.

In Kapitel 3 zeige ich, dass Erwärmung einen größeren Einfluss auf Kopepoden Körpergröße, Abundanzen, Biomasse und die Zusammensetzung von Fettsäuren hat, wobei Versauerung keine signifikanten Auswirkungen auf Kopepoden zeigte. Ich konnte Tendenzen beobachten, die mit einem positiven Effekt durch CO_2 Düngung auf die Phytoplankton-Biomasse einhergehen. Phytoplanktonbiomasse wurde als Stellvertreter von Nahrungsbiomasse verwendet. Es scheint, dass Kopepoden durch höhere Nahrungsaufnahme den negativen Temperatureffekt teilweise ausgleichen können. Die Fettsäurezusammensetzung wurde signifikant durch Temperatureinflüsse verändert. Die Gesamt-Fettsäuremenge veränderte sich nicht durch Temperatur oder Versauerung, aber die Verhältnisse von einzelnen essentiellen mehrfach ungesättigten Fettsäuren zur Gesamt-Fettsäuremenge veränderten sich signifikant. Ich schließe aus meinen Ergebnissen, dass Versauerung das Potenzial zur Abmilderung der Temperatureffekte auf Kopepoden Körpergröße, Abundanz und Biomasse, durch höhere Mengen verfügbarer Nahrungsbiomasse, hat. Kopepodengemeinschaften könnten mehr durch Erwärmung als durch Versauerung alleine beeinflusst werden.

Meine in Kapitel vier präsentierte experimentelle Arbeit, fokussiert sich auf eine mehr Generationen Mesokosmen Studie, das zur Beurteilung von Temperatureffekten auf die metabolischen Raten von Netto-Primär-Produktion und Respiration dient. Eine Abnahme von Phytoplanktonbiomasse, Zooplankton Größe und Abundanzen verstärken, die durch Erwärmung gesteigerten metabolische Raten. Die Ergebnisse zeigen, dass die zukünftige Erwärmung von aquatischen Ökosystemen mehr auf höhere trophische Ebenen wirkt, da sich die Nahrungsnetzstruktur zu geringeren Phytoplanktonbiomassen und Zooplanktonabundanzen mit geringerer Körpergröße ändert. Ich empfehle, dass Biomasseveränderungen mit in die Analyse von Ökosystem Sauerstoffflüssen eingeschlossen werden sollten.

Meine experimentellen Studien zusammenfassend, habe ich ein Schema entwickelt, dass die Temperatureinwirkungen auf die biotische Interaktionen in aquatischen Planktongemeinschaften zeigen. In diesem Diagramm wirkt Temperatur direkt auf Kopepoden über die metabolischen Raten, was indirekt zu kleineren Körpergrößen und geringeren Abundanzen führt, was wiederum zu geringeren Biomassen von Kopepoden führt. Höhere metabolische Raten führen zu gesteigerten Energiebedarf und höherer Fraßaktivität auf Phytoplankton. Die höhere Fraßaktivität beeinflusst indirekt die Phytoplanktongemeinschaften.

Meine Arbeit stellt die Bedeutung von komplexen Gemeinschaftsstudien mit ihren gesamten Interaktionen mit trophischen Ebenen heraus, die zum Verständnis von ökologischen Prozessen in aquatischen Ökosystemen und ihren Reaktionen auf den vorhergesagten Klimawandel führen. Das Testen von mehreren Faktoren und den Auswirkungen auf der Ebene der Physiologie des einzelnen Organismus, könnte zu einer Änderung der Nahrungsnetzeffizient führen, und somit zur Aufklärung von komplexen Reaktionen beitragen.

GENERAL INTRODUCTION

Global change and marine ecosystems

Oceans cover about 71% of the earth's surface, mainly regulate the world's climate, are inhabited by a myriad of species, act as a major food resource and provide a magnitude of ecosystem services. Marine environments are at a risk due to human activities such as pollution, exploitation, and habitat destruction. In addition, the increasing amount of anthropogenic emissions of carbon dioxide and other greenhouse gases have led to acidified oceans which promote a global temperature increase.

Life on earth has always since faced changing conditions on the planet. With respect to climate, organisms had to deal with changing periods of relatively warm and cold temperatures over geological timescales. However, observations of the last century have indicated that global temperatures tend to rise again. Recent studies by Hansen *et al.* (2006, 2010, 2014) show that global land-ocean temperatures have increased by approximately 0.8°C since the beginning of temperature record (late 1880s) (Fig. 1).

The Intergovernmental Panel on Climate Change (IPCC) presented several scenarios of global warming dependent on anthropogenic greenhouse gas emissions, such as carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and halocarbon concentration in the atmosphere. Greenhouse gases have consistently increased since the beginning of industrial revolution in the 1850s and have exceeded by far the natural range over the last 650.000 years. Prior to the beginning of industrialization, CO_2 concentrations varied between 180 and 300 µatm (Siegenthaler *et al.* 2005). As a result of human activity, today's atmospheric CO_2 concentration is 380 µatm and increases at a rate of ~ 0.5% year^{-1} (Forster *et al.* 2007).

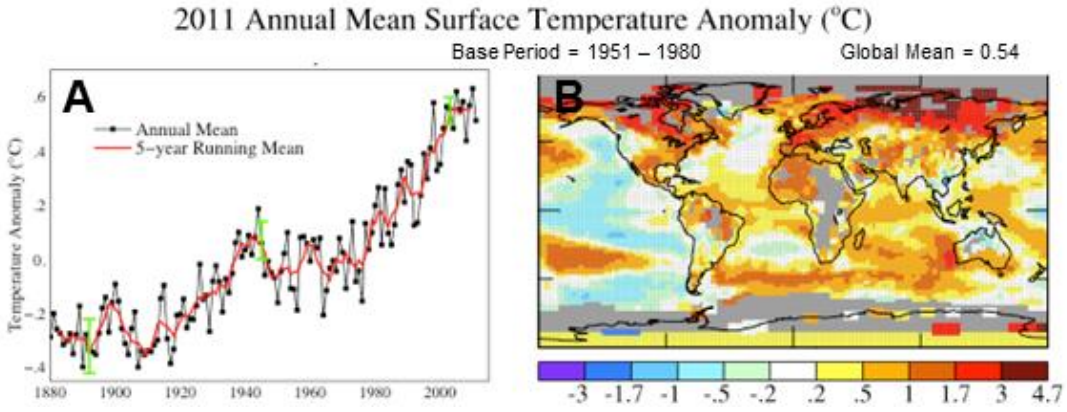


Fig. 1 Surface temperature anomalies relative to 1951-1980 from surface air measurements at meteorological stations, ship, and satellite SST measurements. A) Global land-ocean temperature annual mean anomalies (°C). **B)** Mean surface temperature anomaly for the first half decade of the 21st century (2000-2005). Hansen *et al.* (2006).

Copepods

Ectothermic organisms comprise over 99% of species worldwide. At the turn of the 20th century, Jenkins (1901) pointed out that "the plankton undoubtedly forms the sole food supply for many of our most important food fishes" and that (variation in the production of fishes) is in all likelihood connected with the variation in their food supply, that is, in the variation of the plankton, or more particularly the variation in the copepod constituents of the same". Calanoid copepods often dominate the mesozooplankton in terms of abundance and biomass, comprising as much as 80% of its total biomass in the ocean (Verity & Smetacek 1996), and some species occur over wide biogeographic regions (Mauchline *et al.* 1998). They are generally regarded as a key component in marine food webs and many ecosystems because they are globally distributed from oceans, brackish water to freshwater environments (Mauchline *et al.* 1998). On the other hand, copepods represent the link between phytoplankton and planktivorous predators (e.g. fish larvae) (Atkinson 1996), whereas grazing on primary producers and microzooplankton forms an important link between primary production, the microbial loop and higher trophic levels (Atkinson 1996). Copepods also play a fundamental role in the upper ocean - exporting, redistributing and repackaging carbon and nutrients (Banse 1995).

Warming and acidification predictions

Marine pelagic invertebrates live in a multi-stressor world where stressor levels are, and will continue to be, exacerbated by global change (Cladeira & Wickett 2003; IPCC 2014). Predicting potential impacts of climate change and ocean acidification on marine biota poses a significant challenge for integrative marine biology and ecology (Harley *et al.* 2006; Przeslawski *et al.* 2008). Increasing atmospheric carbon dioxide is altering the levels of co-occurring stressors, resulting in increasing sea-surface temperatures (SST), pCO_2, decreasing O_2, and, due to temperature-related water body stratification, lower nutrient flux to the surface waters.

Future warming for the end of the 21st century has been forecasted to range between 1.1 °C and 6.4 °C, with different probable scenarios. Recent observations confirm increasing sea surface temperature (IPCC 2014). The concentration of atmospheric CO_2 has increased from 280 µatm in pre-industrial times to a present level of 391 µatm (Le Quéré *et al.* 2012). By the year 2100, CO_2 (aq) is expected to further increase, which will result in a pH reduction by 0.3-0.5 units.

Changing nutrient availability

Physically mediated effects of increased SST consist in enhanced vertical stratification and the reduced mixed layer depth, which in turn leads to lower the diapycnal flux of nutrients into the photic zone (Behrenfeld *et al.* 2006; Doney 2006), which results in nutrient-depleted conditions in surface layers (Livingstone 2003; Schmittner 2005). Additionally, the frequency of extreme rainfalls and severe drought has increased since the late 1970s (IPCC 2014), affecting nutrient runoff from terrestrial sources to the ocean (Briceño & Boyer 2010). The increased runoff can also modify the resource ratio in certain types of systems like e.g. shelf seas, which are additionally directly affected through anthropogenic nutrient input.

Increased availability of carbon, as a result of increasing CO_2 concentrations in the environment, may have additional impacts in marine organisms. Primary producers will be directly affected by increased carbon to nutrient availability. Burkhardt *et al.* (1999) showed

that nutrient limitation changes the algae growth rate and cell tissue composition with increased carbon-to-nutrient ratio. The changes of nutrient stoichiometry in phytoplankton has the potential of limiting essential nutrients (e.g. nitrogen and phosphorous), that is affected consequently food quality of herbivorous consumers. Experimental studies of Schoo *et al.* (2013) have shown that acidification has the potential to change C-to-nutrient ratios in algae and consequently has affected copepods, which feed on the algae.

Stoichiometry theory predicts that zooplankton growth and nutrient recycling are tightly coupled with resource nutrient ratios. Consumers should release much of nutrients present in excess, while retaining most of the limiting nutrient. This implies that the quality of a certain resource is determined by its nutrient ratio compared to requirements of the consumer and that homeostasis of consumers presents a useful approach in understanding growth rates.

Phosphorus is an essential nutrient for physiological processes. Phosphate is mostly allocated in cells in form of RNA owing by high demand by ribosomes, protein biosynthesis, nucleic acids, and the regeneration of the universal cell energy carrier from ADP to ATP (Elser *et al.* 1996; Vrede *et al.* 1999). P-limitation is proposed to lower the production of new biomolecules like proteins, amino acids (Sterner & Elser 2002) and decreases the RNA-to-DNA ratio (Malzahn *et al.* 2007). P-limitation effects on aquatic animals have been experimentally investigated with freshwater (e.g. *Daphnia* sp., (Boersma *et al.* 2001; Plath & Boersma 2001), and marine zooplankton organisms (Malzahn *et al.* 2007; Malzahn & Boersma 2012).

Biotic responses to warming

Increasing temperature has direct and indirect effects for marine organisms. Increasing SST has led to an increased stratification of the water column, which reduces the vertical mixing and lowers the nutrient transfer from deeper waters into the surface layers (Behrenfeld *et al.* 2006; Doney 2006). More stratified nutrient limited waters favour small phytoplankton species over larger ones, which require more nutrients (Bopp *et al.* 2005).

A review on all available multi-species studies until 2003 revealed that 41% of the observed species had already responded to climate warming (Parmesan & Yohe 2003). These responses can be roughly grouped into three categories, corresponding to different levels of ecological organization (Walther *et al.* 2002): phenology and physiology (individual level), range and distribution of species (community level), and on structure and dynamics of ecosystems (ecosystem level).

Whole ecosystem consequences can be detected in terms of productivity, stability, and structure. One terrestrial example was presented by Cleland *et al.* (2007), where they observed that shifting plant physiology alters whole system productivity and consequently changes carbon cycling on a global scale. In marine systems, Edwards and Richardson (2004), showed that abating synchronization of successive trophic levels induces mismatched situations, and identifies the decline of economically important fish stocks (Beaugrand *et al.* 2002), which are additionally threatened by overfishing.

Ecosystem regime shifts, meaning that systems are shifting from a steady state to another condition, characterized by completely new structures and functions of subsequent trophic levels. Aquatic regime shifts have been described for lakes (e.g. Scheffer *et al.* (2001)), brackish waters (e.g. Möllmann *et al.* (2008)), and marine systems (e.g. Drinkwater (2006)).

Range shifts are acting on population levels in terms of species range shifts that lead to alterations of zonation patterns or species invasions. Examples for community changes in aquatic systems have observed that arctic lakes changed to more planktonic and warm-water associated communities (Smol *et al.* 2005). In the North Atlantic, the dominance of pico-plankton increased with temperature (Morán *et al.* 2010), or from diatoms to non-silified coccolithophor species (Stockwell *et al.* 2001).

Temperature has a direct impact on physiology of ectotherms because metabolic rates (e.g. enzyme activities) vary with reaction temperature. Metabolism provides the basis for main principles of physics, chemistry, and biology to link biology of individual organisms to ecology of populations, communities, and ecosystems. Metabolic rate, or the rate at which an organism takes up, transforms, and expends energy and material, is the fundamental biological rate. The 'Metabolic Theory of Ecology' postulated by Brown *et al.* (2004), predicts how metabolic rate varies with body size and temperature, and in which ways resources are taken up from the environment and are allocated to survival, growth and reproduction. These basic ecological processes control all levels of organization from individuals to the biosphere: (1) life history attributes, like development, mortality, population growth rates; (2) population interactions like carrying capacity, competition or predation; and (3) ecosystem processes like biomass production rates and community respiration.

Recent research studies have linked metabolic theory to ecosystem metabolism by attempting to establish temperature dependence of fundamental components of the carbon cycle (net primary production and ecosystem respiration)(Yvon-Durocher *et al.* 2010) and further on relating the complex ecosystem-level phenomena to the effects of body mass and temperature on individual-level metabolic rate (Yvon-Durocher & Allen 2012).

Body size changes

Body size is a fundamental biological characteristic that scales with many ecological properties (e.g. fecundity, population growth rate, competitive interaction) (Millien *et al.* 2006; Arendt 2007). The relationship between body size and climate is a classical macro-ecological pattern (Bergmann 1847). Blackburn *et al.* (1999) suggested applying James (1970) for climate related size changes within species, while Bergmann's rule should be used for interspecific trends.

Atkinson and Sibly (1997) concluded in a meta-analysis that 83% of 109 studied ectothermic species have larger body sizes with colder rearing temperatures and had slower developmental rates with decreasing temperature. They called this plastic response of body size "developmental temperature-size-rule" or just "Temperature-Size Rule" (TSR). Later, studies have described ecological responses of declining body size associated with global warming (Daufresne *et al.* 2009; Sheridan & Bickford 2011). Three ecological responses to changing temperatures have been stated: (1) species range shift (Parmesan & Yohe 2003), (2) change in phenology (Walther *et al.* 2002) and (3) TSR that states that the individual body size of ectotherms tends to decrease with warmer temperatures (Atkinson 1994). Daufresne *et al.* (2009) proposed five hierarchical and non-mutually exclusive hypotheses concerning potential effects of climate change on size structures from individual to community scales (Fig. 2). First, they proposed that mean body size at the community scale decreases at warmer temperatures (community body size shift hypothesis). Three subsequent hypotheses could explain this community size decrease. A) Proportional increase of small-sized species (species shift hypothesis) in terms of abundances of

individuals. B) population body size shift due to (1) size-at-stage decrease (size-at-age shift hypothesis) or (2) increase of juveniles at population level (population-age-structure shift hypothesis).

Size changes in natural communities can be attributed to three distinct components i) species shift, ii) shifts in age structure of populations and iii) size shifts at defined age or developmental stage (Daufresne *et al.* 2009) and because physiological theory of body-size dependency on temperature only recently came back into focus (Goodman *et al.* 2012).

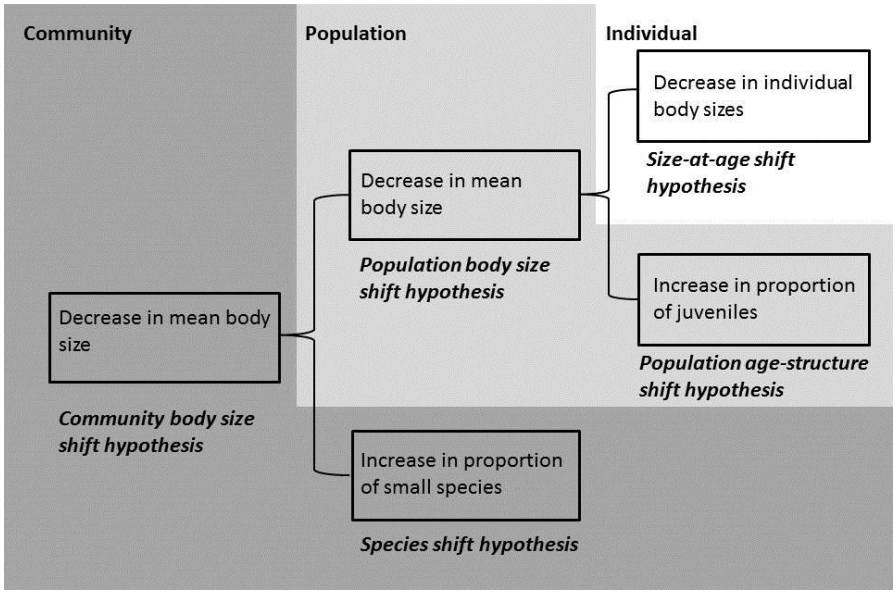


Fig. 2 Hypotheses regarding the impact of warming on body size at different biological scales (according to Daufresne *et al.* 2009).

THESIS OUTLINE

This thesis is divided into four chapters. Each chapter represents an independent study addressing the effects of either temperature, or temperature combined with nutrient limitation or acidification on the body size, phenology or abundance of experimental aquatic zooplankton on community or individual level. This outline gives a brief overview of the motivation for the single experimental studies.

Chapter 1

The first chapter investigates temperature effects at the population level of the Kiel Fjord natural copepod community. The aim of this study was to better understand how temperature changes the response of copepod communities in terms of body size, in particular inter- and intra-specific size-at-developmental stage, age structure, abundance, and on population dynamics. I performed a mesocosm study with 3 temperature levels (ambient, +4 and -4 °C) and the natural late spring Kiel Fjord plankton community. I explored shifts in copepod abundance and developmental stage distribution, prosome length changes of all occurring copepod species and of each occurring copepodit developmental stage. I suspected that temperature indirect effect on copepods by changing metabolism leads to body size decrease at warmer environmental temperature. Secondly, I predicted that food biomass is not driving body size changes of copepods.

Chapter 2

In the second chapter, my main objective was to combine temperature and changes of nutrient supply and their effects on body size, development, somatic growth, and respiration. I performed a microcosm experiment with 5 temperature steps and 3 different carbon-to-phosphorus ratios of the food algae *Rhodomonas salina* and the effects on the copepod species *Acartia tonsa*. Higher water temperatures lead to more stratified water layers and consequently result a lower nutrient flux to upper surface layers, which lead to changes in carbon-to-nutrient stoichiometry in algae. We observed that body size of the two developmental stages (C1 copepodits and adult copepods) decrease with warming but is not directly related to nutrient supply. I could further conclude that the observed decline of body size at higher temperatures is more related to different temperature sensitivities of development and somatic growth.

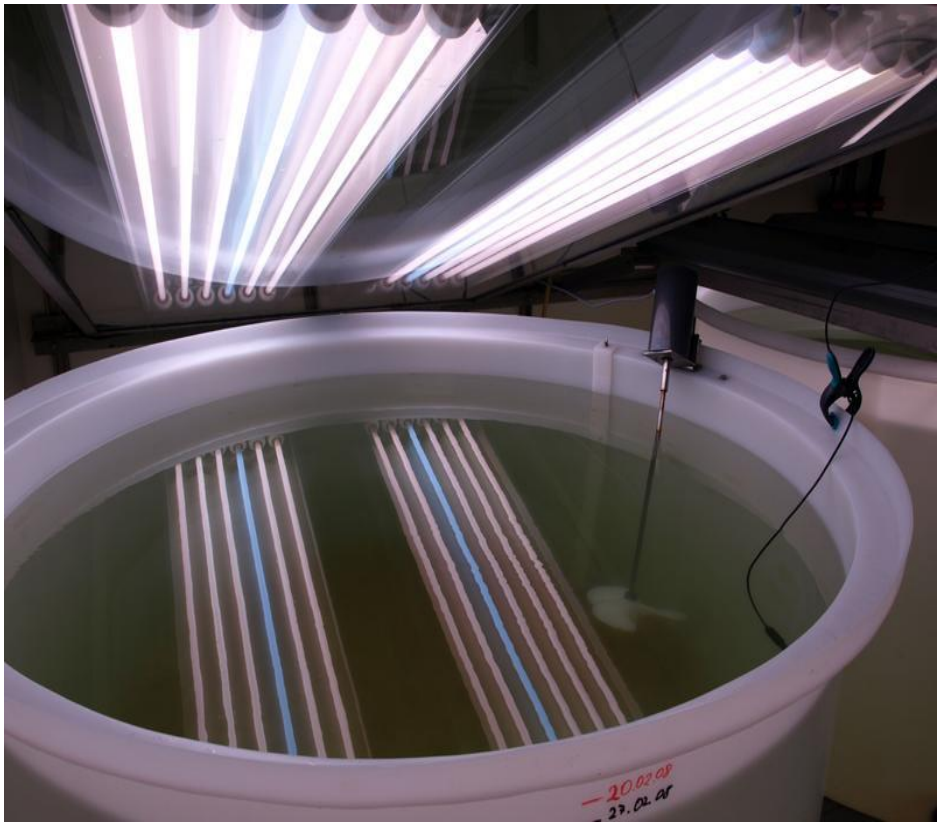
Chapter 3

In the third chapter, I focused on effects of higher temperatures and acidification on copepod communities. I performed a mesocosm experiment with the natural Kiel Fjord plankton community where temperature (9 °C and 16 °C) and acidification (500 µatm and 1000 µatm) were manipulated. I could observe that the chosen temperature regimes had bigger impacts on the copepod community- and individual level than acidification. We observed decreasing body sizes, as in the previous experiments, shifts in population age structure and changes in

fatty acid composition. Generally, higher pCO_2 had a fertilizing effect on phytoplankton, which I used as a proxy of food availability. Higher food availability under high pCO_2 was able to partially compensate the lower abundance and smaller body sizes of copepods. Copepod abundance was higher at high pCO_2 and high temperature, as well as prosome length-at-defined stage of *Paracalanus* sp.

Chapter 4

In the first three chapters I concluded that temperature had a strong effect on zooplankton body size and abundance, whereas other combined factors like nutrient availability and acidification had no significant effects. I assumed that temperature-driven zooplankton and also phytoplankton responses is able to change mass-specific metabolic fluxes of whole ecosystems. Thus the next experiment and chapter 4 focuses on ecosystem net primary production and respiration, and how both metabolic fluxes are changing with warming in respect of changing biomasses. I performed a freshwater mesocosm experiment with a temperature gradient of 10 °C and three different trophic levels (phytoplankton, phytoplankton and herbivore consumers, and phytoplankton, herbivore consumers and predators) over a period of 10 weeks. I predicted that temperature related changes of phytoplankton and zooplankton biomass lead to different ecosystem fluxes with warming and further food chain length is driving to differences of ecosystem metabolism with higher temperatures.



CHAPTER 1

Climate change affects low trophic level marine consumers: warming decreases copepod size and abundance

Abstract

Concern about climate change has re-ignited interest in universal ecological responses to temperature variations: 1) biogeographical shifts, 2) phenology changes, and 3) size shifts. In this study we used copepods as model organisms to study size responses to temperature because of their central role in the pelagic food web and because of the ontogenetic length constancy between molts, which facilitates the definition of size of distinct developmental stages. In order to test the expected temperature induced shifts towards smaller body size and lower abundances under warming conditions, a mesocosm experiment using plankton from the Baltic Sea at three temperature levels (ambient, ambient +4 °C, ambient -4° C) was performed in summer 2010. Overall copepod and copepodid abundances, copepod size at all life stages and adult copepod size in particular, showed significant temperature effects. As expected, zooplankton peak abundance was lower in warm than in ambient treatments. Copepod size-at-immature stage significantly increased in cold treatments, while adult size significantly decreased in warm treatments.

Introduction

Global warming is considered to be one of the most important factors for future changes in marine ecosystems (Boyce *et al.* 2010; Mackas *et al.* 2012) with ocean surface temperatures predicted to increase by 1 to 6 °C within the 21st century (IPCC 2007).

At the basis of the marine food web, elevated temperature is associated with a decline in phytoplankton biomass (Boyce *et al.* 2010; Sommer and Lengfellner 2008; Sommer and Lewandowska 2011). Increasing temperatures are found to correlate with lower mesozooplankton abundances (Edwards and Richardson 2004) and have been experimentally shown to be linked with faster zooplankton die-off (Sommer *et al.* 2007), but experimental assessment of the direct temperature effects on zooplankton morphology, phenology and abundance vs. indirect effects through temperature impacts on the food supply is still missing.

On a biogeographic scale, shifts in species ranges towards higher latitudes and altitudes are the most widespread ecological change ascribed to global warming (Beaugrand *et al.* 2002; Parmesan and Yohe 2003). Those observations are often used to forecast the effects of warming on biota and their future distribution according to climate-change scenarios

(Pearman *et al.* 2008). Besides the shifts in species range, the second widely observed response to warming is a change in phenology (Walther *et al.* 2002).

As a third universal response to warming, rising temperature can lead to a decrease in body size (Daufresne *et al.* 2009; Gardner *et al.* 2011; Walther *et al.* 2002). The relationship between body size and climate is a classic macro-ecological pattern (Bergmann 1847). Bergmann's rule originally states that among endotherms larger bodied animals tend to live in colder environments, but it has been extended to other types of organisms by later usage. Blackburn *et al.* (1999) suggested applying James' rule (1970) for climate related size changes within species, while the term Bergmann's rule (1874) should be used for interspecific trends. The physiological theory of body-size dependency on temperature only recently came back into focus in ecology because of the predictions of global warming (Daufresne *et al.* 2009; Goodman *et al.* 2012). Examples for declining body size with warming have been illustrated in both in terrestrial (Gardner *et al.* 2011; Patridge *et al.* 1994) and aquatic ecosystems (Sweeney *et al.* 1986) and across different animal taxa (Goodman *et al.* 2012; Yom-Tov and Geffen 2011), including insects, crustaceans, fish, amphibians, birds and mammals (Daufresne *et al.* 2009; Reading 2007; Sheridan and Bickford 2011). Opposite responses have been found in some study systems, increasing body size to warming in certain fish, lizards, birds, and mammals (Gardner *et al.* 2011; Thresher *et al.* 2007; Yom-Tov *et al.* 2010). Interestingly, Forster *et al.* (2012) found in their meta-analysis that aquatic species respond with a ten times stronger body mass decrease to warming (-5 % $°C^{-1}$) than found in terrestrial species (-0.5 % $C°^{-1}$) and discuss that O_2 changes tend to be the driver of size changes in aquatic organisms. Climate related size-changes in natural communities can be attributed to the effects of three distinct components: (1) species shifts, (2) shifts in the age structure of populations, and (3) size shifts at defined age or developmental stage (Daufresne *et al.* 2009).

Copepods are the most important trophic link between primary producers and heterotrophic protists, fish and other higher level consumers in the pelagic food web (Möllmann *et al.* 2005), and known to exhibit all three universal responses to warming, with hypothesis 3 to date-being the least investigated. Batten and Walne (2011) and Beaugrand *et al.* (2002) showed zooplankton distribution shifts with warming. Also, positive changes in abundance with temperature shifts were shown by Möllmann *et al.* (2008) and larger shifts in seasonality forward in holoplanktonic organisms (Edwards and Richardson 2004). Lastly, intra-specific changes in copepod body size with warming have been scarcely investigated. The laboratory studies of Escribano and McLaren (1992) combined food quality and quantity with water temperature, and also field observations to show that copepods of the same genus are smaller than relatives in cooler environments (Kobari and Ikeda (2001) in *Neocalanus plumchrus*, and Gaudy and Verriopoulos (2004) in *Acartia tonsa*).

Here we investigate temperature effects on copepod body size intra-specifically in the same environment and across copepodid and adult stages, while controlling for food availability effects.

Materials & Methods

Experimental design

A 28-day experiment was conducted in early summer (June 16th, 2010). A natural plankton community, containing algae, bacteria and protozoa, from the Kiel Fjord was used as experimental system, utilizing nine 1400 L mesocosms in three climate chambers. Additionally, mesozooplankton, mainly consisting of copepods of the taxa *Acartia* sp., were added from wild net catches.

Apart from copepods (adults, copepodids, and nauplii), the zooplankton inoculum contained larval stages of polychaetes, *Balanus* sp., and bryozoa in natural abundances, but these disappeared after 2 weeks. A target copepod concentration of 10 Ind L^{-1} was added on day -3 to each mesocosms in order to mimic natural densities (Behrends 1996). The plankton was gently stirred by a propeller to guarantee homogeneous mixing of the water column without incurring zooplankton mortality (Sommer *et al.* 2007). Temperature was controlled at in-situ values of the Kiel Fjord (13.5 °C) in one climate chamber, and at +4 (17.5 °C) and -4 (9.5 °C) in the other two chambers, mimicking the extent of warming predicted for this region and season (IPCC 2007). A symmetric design of temperature manipulations (warming and cooling) was chosen in order to distinguish effects of the direction of temperature change from effects of change as such. Light supply and day length were adjusted according to the seasonal patterns expected at this latitude.

Zooplankton was sampled weekly by three vertical net hauls, starting on day 0 of the experiment, with hand-held plankton net of 64 µm mesh size and 12 cm diameter. Each net haul sampled a volume of 5.1 L. All samples were fixed with Lugol's iodine. Each zooplankton sample was gently and homogenously mixed, and divided with a zooplankton divider. One fourth of each sample was identified to taxon level, and developmental stage, and if possible, sexes of all copepods were determined. The body size constancy between molts enabled a clear assignment of size to stage. All copepods were identified to genus level by using a ZEISS Discovery V.8 microscope with the magnifications between 2.5x and 4.0x, and whenever possible developmental stage and sex of each individual were recorded. The mean developmental index was calculated after a modified formula used by Villegas and Kanazawa (1979):

DI=A/total number of copepods staged

Where A=∑(assigned stage value × number of copepods at stage) / number of total staged copepods.

Stages were scored as:

C1 = 1, C2 =2, C3 = 3, C4 = 4, C5 = 5, Adult = 6

Because of observed differences in developmental cycles in the different temperature treatments, the population age structures of the last day of the experiment were compared.

Prosome lengths of identified copepods were digitally measured via photographs and digital software (ZEISS AxioVision 4.8 and AxioCam MRc) with a precision to the nearest µm; each magnification and parallel photographs of each magnification were individually calibrated. Means and standard deviations were calculated stage-specifically for copepods of each genus found in each mesocosm.

The diversity of the copepod community in the mesocosms was compared by calculating the Shannon-Wiener Diversity Indices (Shannon and Weaver 1963) for all sampling days and mesocosms. Means of diversity indices of the last sampling day were compared between the different temperature treatments. A fixed effect model was conducted to identify possible changes copepod diversity.

Phytoplankton was sampled in 3 times a week, starting on day 0 of the experiment. 250 mL samples were taken from 10 L bulk water samples. All samples were fixed with Lugol's iodine. Mixed phytoplankton samples were divided into 100 mL sub-samples and phytoplankton > 5 µm were counted by the inverted microscope method (Utermöhl 1958), phytoplankton species were identified to genus level and cell size measured. Edible phytoplankton species were the diatoms *Proboscia* sp., *Nitzschia* sp., *Thalassionema* sp., *Skeletonema* sp. the cryptophyte *Teleaulax* sp., and the ciliates *Strobilidium* sp. and *Strombidium* sp. Total phytoplankton biomass consisted mainly of the species *Dactyliosolen fragilissimus*.

Acartia sp. biomass was calculated according to Gismervik *et al.* (2002), ciliate biovolume was calculated according to Hillebrand *et al.* (1999), size corrected for shrinkage caused by fixation (Müller and Geller 1993) and converted to carbon content (Putt and Stoecker 1989). Phytoplankton biomass was calculated from counts and volume measurements according to Menden-Deuer and Lessard (2000).

Temperature-dependent developmental rates of *Acartia* sp. were taken from Leandro *et al.* (2006) to calculate the loss during the development from nauplii stages to copepodid stages, respectively (Appendix 1). These loss terms were subtracted from the total observed nauplii abundance to calculate the survival rate from one sampling date to the following. The survival rate was calculated for ambient and cold temperature treatments from nauplii to copepodid stage C2. The survival rate for warm temperature treatments was estimated from nauplii to C2 by linear interpolation, because copepodids reached the C5 stage within the 7d sampling interval.

Statistical analysis

The copepod biomass, prosome length and log transformed abundances (n+1) were firstly tested for normal distribution and equal variance applying Kolmogorov-Smirnov tests followed by a Kruskal-Wallis ANOVA on ranks test for temperature effects (α=0.05) in SigmaPlot version 11.0, Systat Software, Inc., San Jose California USA. Subsequently, linear-mixed models were formulated in Rstudio 2012, Version 0.97.551, to investigate temperature effects on copepod biomass, abundance, prosome length and diversity. The linear-mixed models for abundance and prosome length were run for all occurring taxa of copepodids and adults as well as for the predominant taxon *Acartia* sp. (Cottingham *et al.* 2005; Krueger and Tian 2004). Temperature and taxon were set as categorical factors, time in days as repeated measures (continuous).

Abundance

Linear-mixed models for copepod abundance statistics were run for nauplii, adult copepods of all taxa and all developmental stages of the predominant taxon *Acartia* sp. Temperature

and species were set as categorical factors, time as repeated measure and mesocosm ID as random factor.

Edible phytoplankton biomass was analyzed by cross-correlations through time within mesocosms and following ANOVA with correlation coefficients for temperature effects. Additionally, correlation analysis of total phytoplankton and ciliate biomass were measured on the final day of the experiment to examine general trends.

Size

An *Acartia* sp. size analysis was conducted in a linear-mixed model over time. Temperature and developmental stage were set as explanatory categorical factors, and time as a repeated measure. Mesocosm ID was used as random factor.

Size analyses of all adult copepods of every occurring taxon were conducted as linear-mixed models over time. Temperature and species were set as categorical factor, time as repeated measure and mesocosm ID was used as random factor.

Temperature effects on sizes of the last experimental day were analyzed using ANOVA with size means of each species and developmental stage for every mesocosm.

Diversity

The diversity analysis was conducted in a linear-mixed model. The Shannon-Wiener-Index (H') was calculated for each treatment and replicate (Shannon and Weaver 1963). H' was used as a continuous response variable; temperature as a categorical variable and time set as a repeated measure. The mesocosm ID was set as random factor.

All tests were conducted at a significance threshold of $\alpha=0.05$ and with post hoc Bonferroni-corrected Mann-Whitney tests.

Results

Copepod abundance

Total copepodid abundance of all occurring taxa was significantly affected by temperature ($P=0.0358$, $dF= 7$, $n=270$) and time ($P=0.0014$, $dF=69$, $n=255$) as well as the interaction term of temperature and time ($P=0.0353$, $dF=69$, $n=225$, Tab. 1.1, Fig. 1.1). The linear-mixed model results for adult copepods revealed significantly lower abundances in warm than in cold treatments ($P=0.039$, $dF=2$, $n=269$) and with time ($P=0.009$, $dF=1$, $n=254$, Tab. 1.1). The abundance of *Acartia* sp., including all copepodid stages, was lower in warm than in ambient and cold treatments (Fig. 1.2., Fig. 1.3). The highest nauplii abundance was found in cold and the lowest in the warm treatments (Fig. 1.4). Also, nauplii abundance was significantly affected by temperature ($P>0.0001$, $dF=2$), time ($P=0.0000$, $dF=4$) and the interaction of temperature and time ($P=0.0000$, $dF=34$, Tab. 1.1). This represents the reproductive success of all copepod taxa during the experiment.

Tab. 1.1 Linear mixed effect model results of total copepodid abundance changes between temperatures over the course of the experiment (values in bold are significant at $p < 0.05$).

Variable	Factor	SE	t-Ratio	p-value	dF
total copepodid abundance changes	Intercept	60.041	10.805	**<0.01**	33
	13.5°C	4.399	0.214	**0.036**	6
	17.5°C	3.457	0.342	**0.031**	6
	time	0.794	5.574	**<0.01**	33
	13.5°C:time	1.123	-1.458	0.154	33
	17.5:time	1.123	-3.662	**<0.001**	33
adult copepods of all occurring taxa (R^2=0.4604)	Intercept	1.349	-2.220	**0.027**	
	time	0.007	2.620	**0.009**	1
	temperature	0.138	5.823	**0.039**	2
	Species		43.583	**<0.001**	5
	Acartia adult	0.162	14.760	**< 0.001**	4
	Centropages adult	0.164	-2.690	**<0.01**	4
	Oithona adult	0.162	-3.220	**<0.001**	4
	Paracalanus adult	0.162	-3.060	**<0.01**	4
	Pseudocalanus adult	0.162	-2.740	**<0.01**	4
	13.5 – 9.5 °C	0.136	-1.810	0.121	2
	17.5 – 13.5 °C	0.139	-1.610	0.158	2
total abundance nauplii changes between temperatures	Intercept	28.547	0.116	0.908	33
	13.5 °C	40.371	0.538	0.610	6
	17.5 °C	40.371	-0.085	0.935	6
	time	1.665	7.280	**<0.01**	33
	13.5 °C : time	2.355	-3.199	**<0.01**	33
	17.5 °C : time	2.355	-4.103	**<0.001**	33

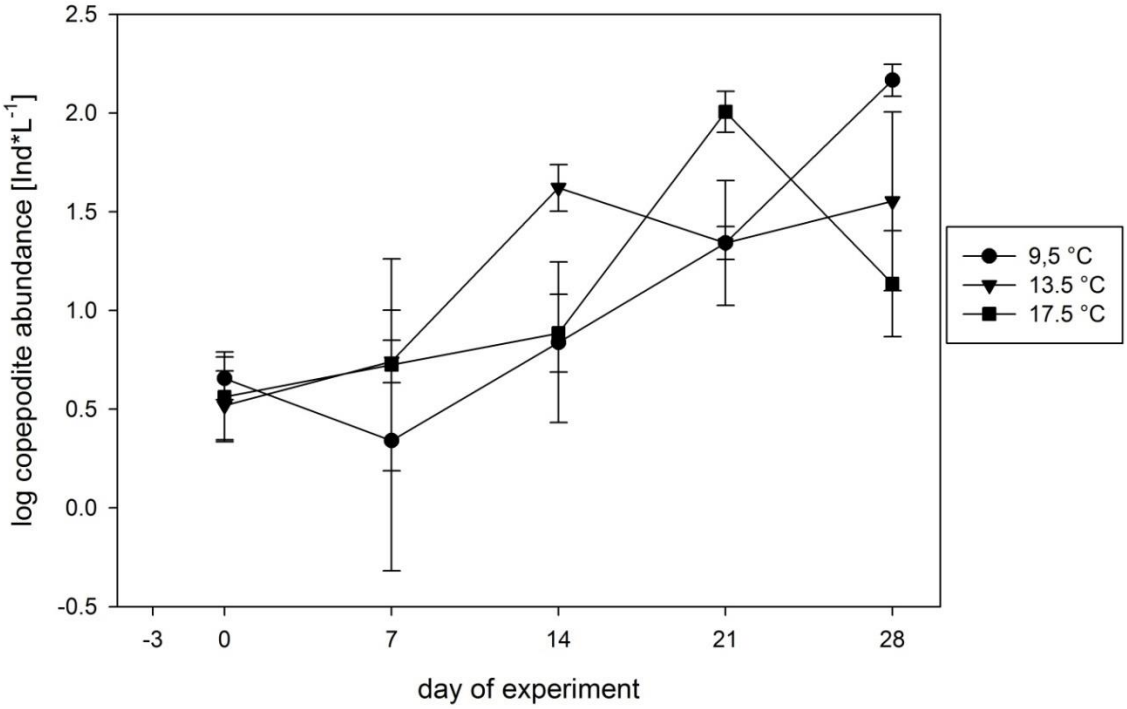


Fig. 1.1 Log abundance (with standard deviation) of all developmental stages of all taxa over the experimental period.

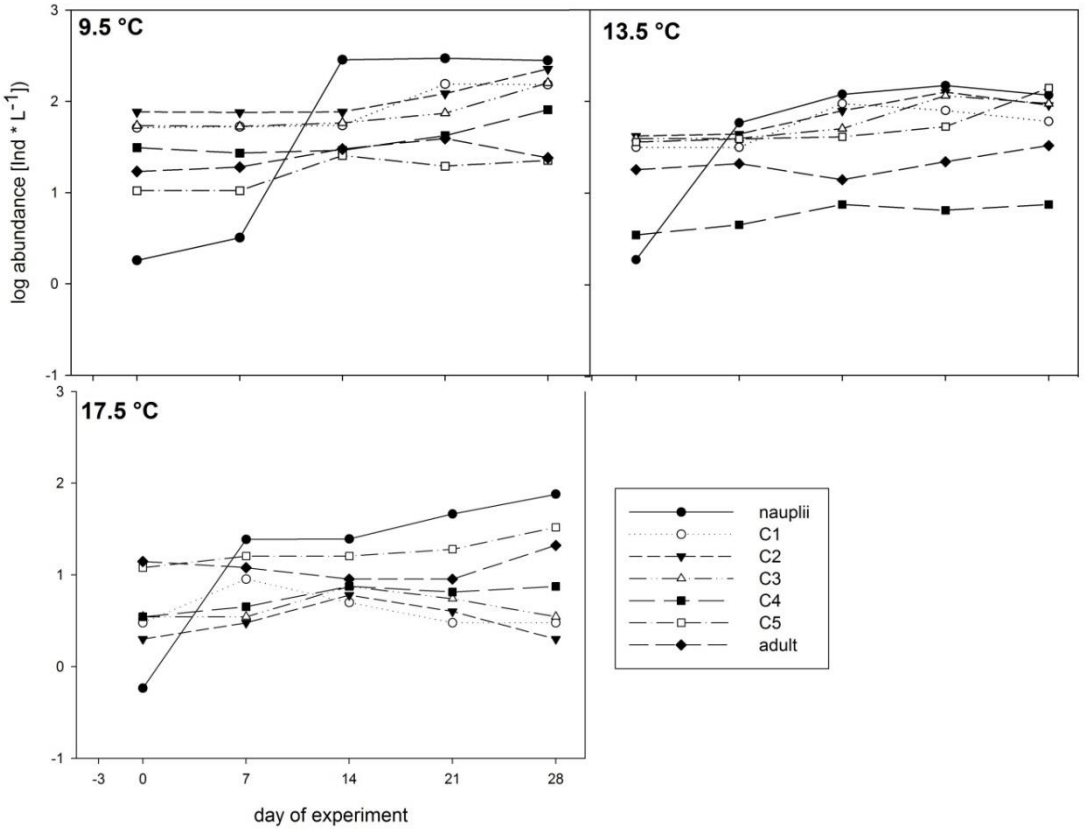


Fig. 1.2 Mean abundance and development succession of *Acartia* sp. over time of all developmental stages (average value with standard deviation).

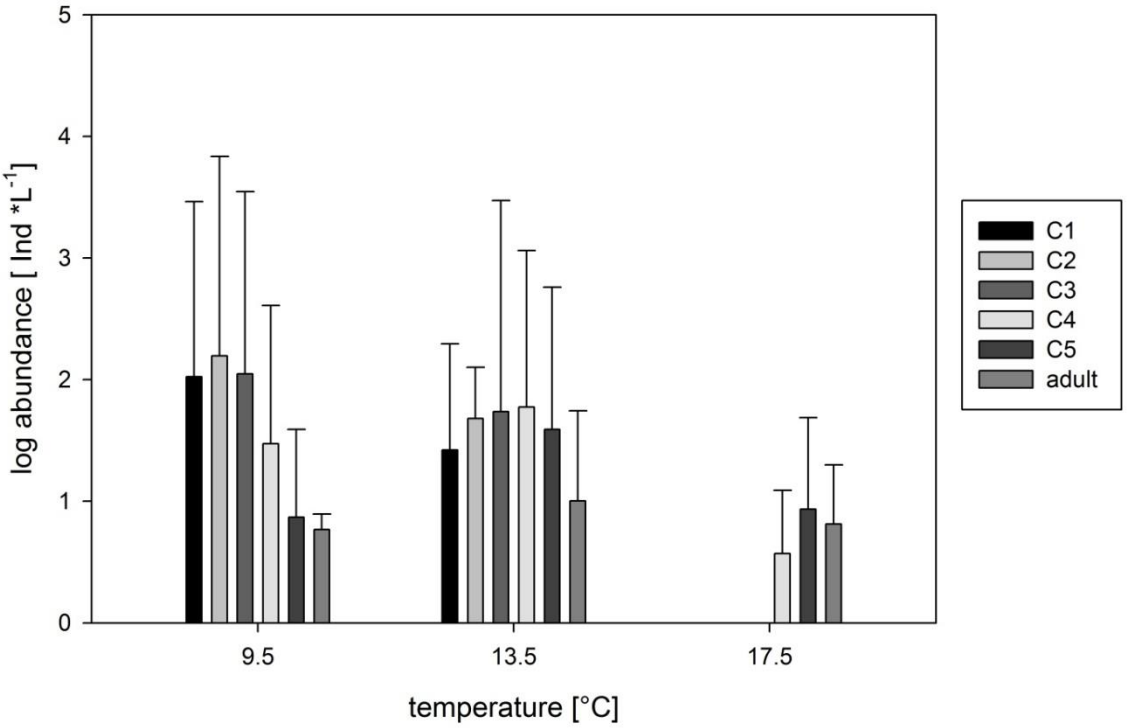


Fig. 1.3 Mean abundances of *Acartia* sp. developmental stages of the last experimental day in all temperature scenarios (average values with standard deviation).

The estimated mean survival rates of the cooling scenario were 14.51 % (± 3.55 SD) and 17.13% (±8.73 SD) in ambient temperature from nauplii to C2 copepodit stage. The estimated mean survival rate of the warming scenario was 12.51% (±6.82 SD) from nauplii to C5 copepodit stage within 7 days (Tab. 1.2). However, the development time within ambient and cold temperature was 7 days from nauplii developmental stage C2, but in the warm temperature treatments the copepodit stage C2 was reached after 2 days (Tab. 1.2). Therefore, the stage specific survival rate in 17.5 °C was higher from nauplii to stage C2 (75.01%) in spite of a lower time, within 2 days, compared to the cold and ambient treatments, where 7 days were needed to develop from nauplii to stage C2. However after 7 days the survival rates were similar among all treatments.

Tab. 1.2 Estimated *Acartia* sp. survival rates (%) per week of new produced and incubated nauplii

Temperature	Day 7- Day 14	Day 14- Day 21	Day 21- Day 28	Mean (SD)
9.5°C		12% (Nauplii – C2)	17.1% (Nauplii-C2)	14.51 (±3.55)
13.5°C		23.3% (Nauplii-C2)	10.9% (Nauplii-C2)	17.13 (±8.73)
17.5°C	7.1% (Nauplii-C5)	10.2% (Nauplii-C5)	20.2% (Nauplii-C5)	12.51 (±6.82)
Interpolation 17.5°C for estimated development time from nauplii to C2	73.5% (Nauplii-C2) day 7 - day 9	74.4% (Nauplii-C2) day 14 - day 16	77.2% (Nauplii-C2) day 21 - day 23	75.01 (±1.95)

Population age-structure shift

The Mean Developmental Index indicated a population age shift between temperature treatments. The warming scenario (+4 °C to in-situ temperature) had a MDI of 5.01, which indicated a dominance of developmental stage C5. The MDI of the in-situ temperature treatments was a mixture between developmental stage C2 and C3 (2.76) and the cooling scenario (4 °C below in-situ temperature) with 2.47, which represent a mixture of the stages C2 and C3. The observed differences of the mean developmental indices show that, at warmer temperature, copepods developed faster (stage C5) than at colder temperatures (C2-C3). This can also be seen from the relative abundances of the different developmental stages at day 28 (Fig. 1.3).

The cross-correlation coefficients of edible phytoplankton and ciliate biomasses, as copepod food sources of the whole experimental period, were negative for all treatments (P=0.452, dF=2, n=9, Appendix Tab. 1.1). The ANOVA analysis of all correlation coefficients over the experimental time and all tanks indicate that food biomass (edible phytoplankton and ciliates) did not significantly vary with temperature (P=0.587, dF=2, n=162, Appendix Tab. 1.1 – 1.5).

Tab. 1.3 Linear-mixed model results for mean prosome size changes over the experimental time (values in bold are significant at $p < 0.05$).

Variable	Factor	SE	t-Ratio	p-value	dF
adult copepods of all taxa (R^2=0.4137)	Intercept	36.795	17.32	**<0.001**	1
	time	0.185	3.33	**<0.01**	4
	temperature		12.849	**<0.01**	2
	species		43.193	**<0.001**	5
	13.5 – 9.5	13.997	-1.91	0.106	2
	17.5 – 13.5	14.088	-3.15	**<0.05**	2
	Acartia sp.	6.293	-8.20	**<0.001**	3
	Centropages sp.	9.687	4.59	**<0.001**	3
	Paracalanus sp.	14.721	-0.56	0.572	3
	Pseudocalanus sp.	13.371	6.14	**<0.001**	3
stage-specific copepod size of *Acartia* sp. (R^2=0.902041)	Intercepts	15.974	46.720	**<0.001**	1
	time	0.069	-17.600	**<0.001**	4
	Temp		10.315	**<0.05**	2
	Stage		3339.001	**<0.001**	6
	Adult	3.907	45.340	**<0.001**	6
	C1	4.365	-40.590	**<0.001**	6
	C2	4.364	25.780	**<0.001**	6
	C3	4.274	-8.480	**<0.001**	6
	C4	3.817	2.640	**<0.01**	6
	C5	3.874	28.870	**<0.001**	6
	Temp 9.5 – 13.5	11.641	-2.800	**<0.05**	2
	Temp 13.5 – 17.5	11.727	-1.720	0.137	2

The negative correlation coefficients indicate that temperature had no direct effect on the food biomass and that copepods of all treatments had comparable biomasses of edible phytoplankton and ciliates available over the course of the experiment. Additionally, we performed correlation analyses of food biomasses with adult *Acartia* sp. biomasses, which indicate that food biomass in ambient (R^2=0.949) and warm (R^2=0.970) temperature treatments were affected positively by adult *Acartia* sp. biomasses on the last experimental day (Appendix Tab. 1.4). Yet, these effects did not differ between warm and ambient treatments Edible phytoplankton and ciliate biomass of the cooling scenario was highly negatively correlated with adult *Acartia* sp. biomass (R^2=-0.994, Appendix Tab. 1.4). Also, analysis of variance of the correlation coefficients between food (edible phytoplankton and ciliates) and adult *Acartia* sp. biomasses show no temperature dependent-difference (Appendix Tab. 1.5). Consequently, no significant effect of food availability on adult *Acartia* sp. could be identified (Appendix Tab. 1.5).

Tab. 1.4 ANOVA results for mean prosome length changes of last experimental day (values in bold are significant at $p < 0.05$).

Variable	Factor	Sum Sq	Mean Sq	F-value	p-value	dF
adults (R^2=0.946801)	Temperature	30293	30293	8.958	**<0.01**	1
	Species	142547	35637	10.538	**<0.001**	4
	Temperature:species	6407	2136	0.632	0.605	3
***Acartia* sp. development stages (R^2=0.9945)**	Intercept	3.032	154.19	377.489	**<0.001**	16
	temperature			100.237	**<0.001**	2
	stage			869.414	**<0.001**	5
	temperature*stage			13385.4	**<0.001**	9
	13.5 °C	-7.496	-2.18		**<0.05**	1
	17.5 °C	-24.750	-4.41		**<0.001**	1
	C1	-195.563	14.17		**<0.001**	
	C2	-110.082	-20.03		**<0.001**	
	C3	-52.588	-11.13		**<0.001**	
	C4	24.585	5.54		**<0.001**	
	C5					
	adult	202.813	45.7		**<0.001**	
	13.5 °C * C1	16.909	1.23		0.229	
	13.5 °C * C2	-1.704	-0.25		0.803	
	13.5 °C * C3	-0.732	-0.12		0.906	
	13.5 °C * C4	-7.621	-1.72		0.096	
	13.5 °C * C5					
	13.5 °C * adult	9.901	1.66		0.106	
	17.5 °C * C1	-6.678	-0.26		0.8	
	17.5 °C * C2	34.083	3.33		**<0.01**	
	17.5 °C * C3	5.939	0.69		0.494	
	17.5 °C * C4					
	17.5 °C * C5					
	17.5 °C * adult	-38.028	-4.79		**<0.001**	

Size

The mean prosome length of adult copepods of all occurring taxa significantly decreased over time (P=0.001, dF=4), and female adult copepods significantly decreased more strongly than males (P<0.0001, dF=2, Tab. 1.3). All adults of the occurring taxa had significantly smaller prosome lengths at warmer temperatures (P=0.007, dF=2) than at colder temperature over the course of the experiment (Tab. 1.3). Copepod size analysis of the final experimental day also showed that adult copepod prosome lengths of all taxa, decreased significantly with warming compared to adults at colder temperatures (P=0.0086, dF=2, n=46, Tab. 1.4).

Adult *Acartia* sp. mean prosome length was significantly smaller with warming (P=0.0118, dF=2), and over time (P=0.0001, dF=4, Tab. 1.3). However, on the last experimental day the mean prosome length of *Acartia* sp. across all stages was significantly larger in the cold treatments (P=0.032), but did not show a further significant decrease between warming and ambient temperature (P=0.137, Tab. 1.4). *Acartia* sp. mean prosome length change results of the last experimental day showed a significant decrease with warming (P<0.0001, dF=2, Tab. 1.4). The effect of smaller prosome size with warming was bigger between the warmest and coldest temperature treatment (P=0.0001) than between ambient and warming temperature treatment (P=0.0369, Tab. 1.4). There was no significant temperature effect on mean prosome length over the experimental period between ambient and warming treatments. Adult *Acartia* sp. mean sizes showed stronger relative decrease with temperature than juvenile stages (Tab. 1.4, Fig. 1.6). In contrast to the significant size increase of all copepod developmental stages with cooling, the mean prosome length of adult copepods of all taxa was significantly smaller in warm treatments compared to the ambient temperature (Tab. 1.3, Tab. 1.4). However, in the adult *Acartia* sp. the size change mounted to 123 µm (16.89%) across the temperature gradient of 8 °C showing that particularly warming lead to a bigger relative size change (16.86%) than cooling (7.79%, Appendix Tab. 1.6).

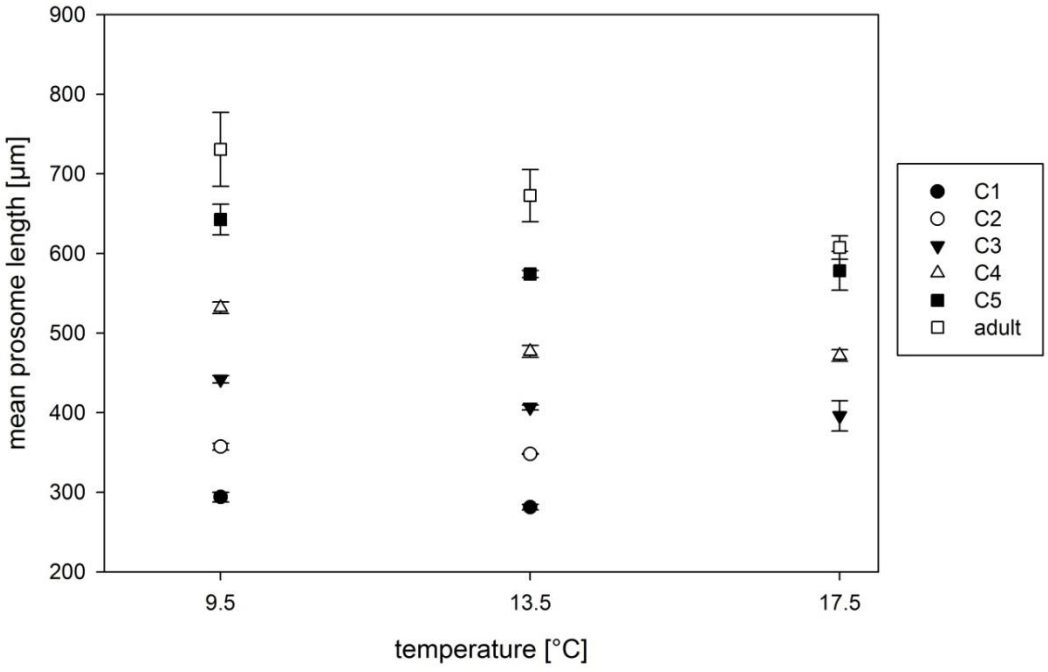


Fig. 1.4 Prosome length (average values with standard deviation) of *Acartia* sp. on the last day of the experiment for all temperature treatments.

Copepod taxonomic composition

The copepod community included the genera *Acartia* sp., *Centropages* sp., *Oithona* sp., *Paracalanus* sp., and *Pseudocalanus* sp. The initial dominance of *Acartia* sp. became stronger in the ambient and cold treatments, while it was slightly reduced in the warm treatment (Appendix Fig. 1.1).

Only time had a significant decreasing effect on Shannon-Wiener indices as a measure of diversity, while diversity of copepod communities did not depend on temperature in our set-up, reflecting a marked dominance of *Acartia* sp. across all temperatures (Tab. 1.5).

Tab. 1.5 Linear-mixed model results of diversity changes in copepods and copepodids (R^2=0.213; values in bold are significant at $p < 0.05$).

Factor	SE	t-Ratio	p-value	dF
Intercept	0.789	2.60	**<0.05**	
time	0.004	-2.16	**<0.05**	1
temperature	0.067	1.524	0.292	2

DISCUSSION

Abundance and Population-Age-Structure Shifts

Observed effects on zooplankton with warming include a decline in total abundance of nauplii, copepodits, as well as fewer adult copepods with warming and a simultaneous shift from a copepodit to a nauplii-dominated population structure. This can be explained by (1) accelerated hatching process of copepods with rising temperature, (2) lower reproduction or (3) higher mortality of copepodits at warmer temperatures. Not only egg production and hatching of copepods are affected by rising temperature, also earlier reproduction with increasing temperature can be expected (Kordas *et al.* 2011). Klein Breteler and Schogt (1994) found that copepods have a higher daily mortality with increasing temperature than copepods in colder environments. This leads to the suggestion that the stage-specific survival rate might be higher while development is slower in colder temperatures and vice versa for copepods in warm temperatures during the present study. An increasing daily mortality at warmer temperatures can be partially, fully or over-compensated by a faster development time at warmer temperatures because of a faster transition from stage to stage (Campbell *et al.* 2001; Hirst and Kiorboe 2002; Leandro *et al.* 2006). This is in agreement with the estimated survival rates in this study. The daily survival rate within the sampling period of one week is slightly lower in warmer than in colder temperatures. Lower copepod daily mortality and higher food availability, phytoplankton biomass (Sommer and Lewandowska 2011), leads to the suggestion that the relative survival rate of nauplii is higher at colder than at warmer temperatures. Not only the faster turnover of generation at warmer temperatures has to be considered, also the direct effect of warmer temperature on the individual metabolism is suggested for changes in population ecology. Brown *et al.* (2004) stated within the ‘Metabolic Theory of ecology’ that faster metabolism at warmer temperatures is able to change the energy demands of the individual organism. A higher metabolic rate might lead to higher energy demands, which have to be covered by higher

feeding rates on the copepods food source phytoplankton and ciliates. The experimental results showed that food availability, here edible phytoplankton and ciliate biomass, did not significantly differ between the temperature treatments neither over the course of the experiment nor on the last experimental day. But the correlations of food and adult *Acartia* sp. biomasses showed that at colder temperatures food biomass declined with higher adult *Acartia* biomass. This might occur because larger bodied adult *Acartia* were more abundant than less and smaller bodied adult *Acartia* at warmer temperatures for similar food source amounts. This may be taken to indicate that larger *Acartia* sp. adults in the colder treatments were able to more efficiently graze the available food. Top-down control was thus only executed of copepods in the cold treatments, while in ambient and warm treatments copepod biomass was bottom-up regulated on the last experimental day. Because of opposite direction of correlation between phytoplankton and ciliate biomass on adult *Acartia* sp. biomass in the different temperature treatments, there is no significant effect shown by our results. However, within temperature treatments our results uncover grazer and food biomass dependencies.

A predominance of mortality effect would be manifested in a lower density of calanoid copepods and nauplii, as shown by a mesocosm experiment in the warm-temperate coastal North Atlantic Ocean by O'Connor *et al.* (2009) and also in this present experiment at the cold-temperate Baltic Sea coast. Hirst and Kiorboe (2002) found that predation mortality contributes an ambient temperature-independent proportion of 2/3 to 3/4 to total mortality. However, predation can be excluded as a result for mortality changes between the different temperature treatments in this study.

Size

Reduction of prosome length in adult copepods could be a result of increasing metabolic demands with increasing temperature. Brown *et al.* (2004) postulated in their Metabolic Theory of Ecology that smaller organisms have faster metabolisms because of basic biochemical and physiological laws. For increasing energy demands to maintain a basal (for staying alive and healthy) and active metabolism (growth and reproduction), it becomes necessary to find a higher quantity or higher quality of food (Claireaux and Lefrancois 2007). Earlier mesocosm studies of Sommer and Lengfellner (2008) showed that the biomass and mean cell size of phytoplankton decreased with increasing temperature. These observations could lead to the conclusion that energy demands of higher metabolic rates cannot be sufficiently fulfilled because of a loss of phytoplankton in biomass and cell size (Brown *et al.* 2004; Lewandowska and Sommer 2010). Biomass data of our experiment did show that food biomass was not directly affected by temperature but that with increasing temperature the correlation of copepod and edible phytoplankton biomass changed. Warming increased the grazing rate of copepods on phytoplankton due to the need of covering the increased energy demand at the warmer temperatures. Stoichiometry analysis of the bulk water components, including all phytoplankton species (edible and non-edible), ciliates and bacteria, showed that the C:N ratio in particulate organic matter increased with increasing temperature (Taucher *et al.* 2012). These differences in C:N ratio might be a hint for lower protein content, higher lipid or carbohydrate content. However, we cannot exclude that maturation at lower size is a genetically fixed adaptation to higher food demands at higher temperatures. We can also support the model of Forster *et al.* (2011) that early life cycle stages show a less pronounced effect in size reduction than adults. The observed *Acartia* sp. stage C1 had

10 µm smaller prosome lengths at 13.5 °C than the mean prosome length of C1 *Acartia* sp. copepodits within the cooling scenario with 9.5 °C. The prosome length difference between the temperature treatments increased with increasing developmental stage. Also in the other taxa a declining cell and body sizes could be observed. The phytoplankton, as in other studies, showed a decreasing average cell size and effective particle size (Sommer, pers. comm.).

One additional factor, which also affects the food web (phytoplankton – zooplankton), is the change of biogenic carbon flow with rising temperature (Wohlers *et al.* 2009). Change in biogenic carbon flow results from changes in size (algae and copepods) and relative abundant activities of heterotrophic nanoflagellates, ciliates and copepods. This observation could show changes in size of algae and copepods as well as in heterotrophic nanoflagellates could reduce the transfer of primary produced organic matter to higher trophic levels. Wohlers *et al.* (2009) observed higher community level respiration with increasing temperature. The combination of a decreasing transfer of organic matter to higher trophic levels, the need of more energy for the basal metabolism of individuals and the decreasing size of organisms can lead to a big loss of energy for copepods. Thus, the biomass of algae as prey for copepods decreases with temperature and, in addition, the cell size of algae is also decreasing. These observations lead to the opinion that smaller algae are more favorable for heterotrophic protists than for copepods. Warmer temperature might have the potential to decrease the energy transfer through the food web, which could result in a re-structuring of the food web to system dominated by smaller-sized species (e.g. protists) (Lewandowska and Sommer 2010). Dominance of smaller sized individuals at warmer temperatures may result in the loss of trophic links herbivorous and carnivorous consumers (Petchey *et al.* 1999; Winder and Sommer 2012). A loss in accessible energy for higher trophic levels like fish larvae might lead to a decrease of these higher trophic levels, e.g. loss in fish abundance or lower reproduction rates. Bochdansky *et al.* (2005) found that fish larvae have higher mortality with food limitation in combination with higher metabolic rates. This would mean that temperature would lead to higher metabolism in fish and their larvae, predicted by the metabolic theory (Brown *et al.* 2004), and would be influenced directly by temperature but also indirectly by limited food sources due to decreasing copepod abundance.

Interestingly, the results show that warmer temperatures significantly decrease the mean population size by causing higher abundance ratios of nauplii-to-copepodids and, additionally, by causing smaller stage-specific sizes. Thus, our results could have consequences for food availability to higher trophic levels, like fish larvae. Less available food with smaller prey may lead to food limitation of favored size classes to cover the needs of copepod-depended higher trophic levels. While it is known that differences in food quality can lead to slower development, smaller body size, and reduced egg production in copepods (Klein Breteler *et al.* 1990; Koski *et al.* 1998). In our experiment we did not assess temperature driven quality changes for phytoplankton and ciliates as a food sources for copepods, such as differences of fatty acid content or carbon-to-nutrient ratios.

The observed and expected patterns of decreasing copepod body size with higher temperature are in line with widely found trends exhibited by different taxa in terrestrial and aquatic ecosystems, spanning from insects and crustaceans, to birds and mammals (Sheridan and Bickford 2011).

Consequences of these temperature-induced body size shifts are likely to alter prey spectra, food availability and feeding efficiency for higher level predators, and thus impact on nutrient transport to and biomass built-up at higher trophic levels.

Future research to identify possible changes in the marine planktonic food web could involve more factors that are predicted to change. Ocean acidification might additionally influence the mesozooplankton communities in the world oceans and might enhance the effects on food web structure. To be able to predict possible changes to the marine food web due to global change is important to estimate future effects, and their implications for ecosystem services.

CHAPTER 2

Interacting effects of nutrient limitation and temperature on size and growth rate of the copepod *Acartia tonsa*

Abstract

Concerns about global warming and temperature-caused changes of nutrient fluxes in water column stratification have re-ignited the interest in studying the universal ecological responses of individual organisms to temperature. Here, we present experimental work assessing how different temperature (from 10 to 20 °C) and nutrient (phosphorus) limitation affects growth of the copepod *Acartia tonsa*, as well as the interaction between these two factors. An increase in temperature significantly reduced the individual body size of the herbivore consumer, whereas phosphorus limitation had no influence. Higher carbon-to-nutrient ratios of the food and increasing temperatures both significantly affect developmental rates and respiration. Phosphorus limitation exacerbated the temperature effect by decreasing developmental rates. A continued increase in temperature and P-limitation based on stronger stratification might accelerate growth rates because of the stronger effects of temperature compared to P limitation.

Introduction

Global warming is one of the most important factors changing marine planktonic ecosystems (Boyce *et al.*, 2010, Lewandowska *et al.*, 2014), with ocean surface temperatures predicted to increase by 1 to 6 °C within the 21st century (IPCC, 2014). An increasing temperature of the ocean surface can have multiple effects on planktonic communities. Obviously, changing temperatures directly affect metabolic rates (Eppley, 1972), an effect typically differing between different players in the community, with potentially varying reactions in phenology and as consequence mismatch phenomena (Durant *et al.*, 2007). Furthermore, warming physically affects the seasonal temperature development of the upper-ocean with consequences for vertical stratification, which may affect phytoplankton by changing nutrient supply rates.

These physically mediated effects of temperature are primarily associated with enhanced vertical stratification and reduced mixed layer depth, which lead to a reduced nutrient flux into the upper oceans (Behrenfeld *et al.*, 2006, Boyce *et al.*, 2010, Doney, 2006). During the last 30 years, a decreasing trend of phosphorus concentration was observed by Wiltshire *et al.* (2008) in the North Sea. Moreover, they observed an annual increase of mean temperature of 0.043 °C. Rising atmospheric levels of CO_2 are predicted to continue to increase the global mean temperature (Houghton, 1996), which also, as a result of the uptake of the CO_2 by the oceans impacts the ocean's climate-buffering capacity due to the impact on the global carbon cycle (biological CO_2-pump) (Schiermeier, 2006).

Changes in environmental temperature affect aquatic and terrestrial organisms in many different ways. Daufresne *et al.* (2009) proposed that body size changes have to be considered as a universal response to global warming. Laboratory studies have shown that an increase in environmental temperature causes a decrease in adult size in the majority of ectotherms (Atkinson, 1994, Atkinson, 1995). Atkinson (1994) formulated the temperature-dependent phenotypical and plastic response of smaller-sized mature organisms in the 'Temperature-Size-Rule' (TSR). A reduction of body size is discussed to be a result of increasing metabolic demands with increasing temperature within the 'Metabolic Theory of Ecology' (MTE) by Brown *et al.* (2004). Brown *et al.* (2004) invoked the idea that basic temperature-dependent principles of the individual metabolism can be extended to population and community ecology and changes, so that temperature-dependent individual changes lead to effects on population and community level. With the increasing body of research on TSR and MTE two possible reasons are discussed for decreasing body size at warmer temperatures. On the one hand, van der Have & de Jong (1996) proposed that growth and developmental rates of ectotherms are differently temperature sensitive, which they showed with a proximate, biophysical model. They argued that development depends on enzymatic regulated DNA replication and cell division, whereas growth depends primarily on the rate of protein biosynthesis, and is limited by diffusion. On the other hand, Forster *et al.* (2011) conducted a meta-analysis of growth and developmental rates across all life stages and found that growth and development rates have significant different temperature dependences across all life stages. They argued that earlier life stages are more temperature sensitive than adults, which may result smaller body size at warmer temperatures.

Further on, higher water surface temperatures and resulting increase of ocean surface stratification have the potential to decrease the nutrient flux (Boyce *et al.* 2010), phosphorus availability, and increase carbon availability, which might have effects on the base of the food web by changing the phytoplankton C-to-nutrient stoichiometry. But also an increasing availability of carbon as a result of the increased atmospheric CO_2 concentrations in the environment may have additional impacts on marine organisms and will be affected directly by higher carbon availability. Higher carbon availability for phytoplankton has the potential to result in limitations in essential nutrients, such as phosphorus and nitrogen with the concurrent change in their food quality for herbivorous consumers (Rossoll *et al.*, 2012, Schoo *et al.*, 2013). An increase in phytoplankton carbon-to-nutrient ratios was experimentally observed and algae with higher carbon-to-nutrient ratios are generally of lower food quality for herbivorous consumers (DeMott, 2003, DeMott *et al.*, 1998, Malzahn & Boersma, 2012).

For most heterotrophic consumers nutrient limitation of the food means an higher amount of carbon is ingested per unit nutrient and to maintain the homeostasis of most consumers, this carbon excess needs to be either egested or evacuated by (1) excretion (DeMott *et al.,* 1998) and/or (2) respiration (Darchambeau *et al.*, 2003, Demott & Tessier, 2002). Independent of the carbon excretion pathway, the surplus of carbon and as a consequence the limitation of elemental nutrients like phosphorus has major consequences for the consumers of herbivores as well as for higher trophic levels like fish (Boersma *et al.*, 2008, Malzahn *et al.*, 2007). Phosphorus is an important nutrient, which is needed for physiological processes. It is allocated in RNA owing to the high demand by ribosomes, RNA synthesis and further for protein synthesis, as well as the regeneration of adenosine triphosphate (ATP) as universal energy transfer (Elser *et al.*, 1996) and nucleic acids (Vrede *et al.*, 1999).

It has been proposed by Sterner and Elser (2002) that lower P availability leads to lower ribosome and RNA production and somatic growth (Boersma, 2000).

Very little is known about the interaction effects between food quality and temperature changes on individual organisms. Dobberfuhl and Elser (2000) observed that cladocerans (*Daphnia* sp.) collected from Arctic lakes had higher C:P ratios than those taken from temperate lakes. They explained this using the growth rate hypothesis: because temperature affects growth rates, high temperatures result in high growth rates and hence a higher P-demand. Based on this we would expect a higher P-requirement at higher temperatures also in copepods, and thus the copepod *Acartia tonsa* in our study to be more affected by high C:P food at high temperatures (see also Persson *et al.*, 2011). Alternatively, in ectotherms higher temperatures increase metabolic rates typically leading to higher respiration rates. Thus, at higher temperatures we would expect a higher C-demand relative to P to deal with the increased respiration rates, provided, of course, that not all of the other rates increase in an identical way. Based on this, we would expect animals at higher temperatures to be less vulnerable to high C:P food. Our working hypotheses are: (1) developmental rate and somatic growth rate are affected by food quality and warming (2) warming decreases copepod body size. In this study, we present experimental results where we combined temperature induced smaller prosome sizes, faster developmental and somatic growth rates of copepodit and adult developmental stages. Additionally, we investigated how the combined manipulation of temperature and nutrient availability affects the three growth parameters.

Materials & Methods

In order to investigate temperature and food quality effects on copepod growth rate, body size and respiration, we cultured the copepod species *Acartia tonsa* in a temperature gradient of 10 °C (10 - 20 °C), using a temperature gradient incubator (Thomas *et al.*, 1963). These temperatures are well within the ecological temperature optimum for our study species (Holste & Peck, 2005). We further tested three different food qualities at all temperatures. The three food quality treatments included P-replete, P-limited and *Rhodomonas salina* that were cultured under P-limited conditions and were pulsed with dissolved PO_4 just before feeding to the copepods (P-pulsed). There are several methodological problems when studying the consequences of P-limitation experimentally because limited algae are able to take up dissolved phosphorus very rapidly but the changes in biochemical composition lag behind (Boersma, 2000, DeMott *et al.*, 1998, Lehman & Sandgren, 1982, Rothhaupt, 1995). The addition of phosphorus to P-limited algal cultures was carried out to test if copepods are able to synthesize molecules from added inorganic phosphorus, which was not assimilated by algal cells as ATP, RNA or phospholipids prior to feeding (Elser *et al.*, 2001, Müller-Navarra, 1995). What exactly drives food quality, and whether phosphorus is the direct limiting factor or a proxy for other biochemical components is still hotly debated (Breteler *et al.*, 2005, Geider & La Roche, 2002). Primary producers that experience phosphorus limitation typically also change their fatty acid content, as well as the composition of the cell wall (Breteler *et al.*, 2005, DeMott *et al.*, 1998). Pulsing nutrient limited algae with phosphorus just before feeding avoids this problem as the algae are biochemically still P-limited, but they contain normal amounts of phosphorus, as uptake from dissolved P-sources is very rapid (Plath & Boersma, 2001). Thus it allows the differentiation of the factors that influence food quality.

For the experiment, we used five replicates per temperature treatment and food source. Each temperature and food treatment was sampled at pre-defined developmental stages C1 and adulthood. Nauplii of age (24-48 h) were sorted into the treatments, and subsequently cultured to C1 stage or adult stage respectively, thus yielding a total of 2 (stages to be harvested) * 5 (temperatures) * 3 (food types) * 5 (replicates) = 150 experimental units.

Phytoplankton

A stock culture of the cryptophyte *R. salina* was cultivated in f/2 enriched seawater (salinity ~32) after Guillard and Ryther (1962). *R. salina* was constantly aerated and cultivated at 18 °C under a 16:6 hours (light:dark) light regime. The seawater used for growth medium was collected prior to the experiment in one single effort from Helgoland Roads on January 15th, 2012, sterile filtered (sterile 0.2 µm filter), and enriched with the full set of nutrients of the f/2 growth medium (8.82 x 10^{-4} mol $NaNO_3$ L^{-1} and 3.63 x 10^{-5} mol NaH_2PO_4 L^{-1}) for P-replete treatments. The filtered seawater was stored cool and dark until use. The algae of two of the phosphorus levels initially grown on 8.82 x 10^{-4} mol $NaNO_3$ L^{-1}; P-pulsed algae were enriched after Guillard and Ryther (1962) with $NaNO_3$. The algae therefore only utilized the natural P-sources present in seawater at the moment of filtration for the low P treatment, which was approximately 0.7 µmol P L^{-1} on the sampling day. For phosphorus pulsed food, phosphorus deficient *R. salina* was enriched with Na_2POH_2 and exposed for 10 minutes prior feeding to the copepods following Schoo *et al.* (2014).

To ensure constant food quality, new cultures of each of the nutrient treatments were inoculated every day with roughly 0.2 10^6 cells mL^{-1} for nutrient replete treatments (P-replete) and 0.5 10^6 cells mL^{-1} for the P manipulated treatments. Algae were harvested at densities of approx. 1.5, and 1.2 10^6 cells L^{-1} (P-replete and –limited, respectively) after a predefined growth phase of four days. Algal carbon to phosphorus contents were measured 9 times during the experiment, and the molar C:P ratio of P-replete algae averaged 245 (SD=2.8), whereas the C:P-ratio of limited algae was 659 (SD=72.5). The P-pulsed algae took up the dissolved phosphorous rapidly and showed a C:P value of 270 (SD=19.3) after 10 minutes (Tab. 2.1, Tab. 2.2)

Tab. 2.1 *R. salina* carbon-to-phosphorus ratio (in mol)

Food quality	C:P (SD)
P-replete	245.82 (42.81)
P-limited	659.30 (72.59)
P-pulsed	270.64 (19.32)

Copepods

Eggs of the calanoid copepod *A. tonsa* that were previously harvested and stored at 4 °C, were incubated in 5 L cylindrical beakers in filtered seawater. The eggs were kept at 18 °C at a 12:12 light cycle until hatching to the first nauplii stage, between 24h and 48h after starting the incubation to minimize variation in stage distribution. The hatched nauplii were separated from the remaining unhatched eggs and divided into 100 mL glass containers at densities of 12 individuals mL^{-1}. The high starting densities were necessary to yield enough material for

analysis after incubating the copepods. We first fed the nauplii after 24 h, assuming that at that point the second naupliar stage was reached when *A. tonsa* starts feeding (Landry, 1983).

Copepods in all treatments were fed 10,000 cells per individual and day corresponding to ~1 mg C L^{-1} day^{-1}, which is considered to be ad libitum feeding (Malzahn *et al.*, 2007, Schoo *et al.*, 2009). Daily water changes were conducted by transferring the animals prior to feeding to containers with new artificial, N- and P-free seawater that was adjusted to a salinity of 30 (salt: hw-Marinemix, www.hw-wiegandt.de). Copepods were harvested when the majority of the animals reached the desired developmental stage. We chose the first copepodit stage (C1) and the adult stage as these endpoints, to identify different sensitivities of young copepodids and adult copepods to each treatment. After harvesting, part of the animals was collected and the stage of at least 100 animals was determined for estimation of developmental rate. Furthermore, 30-50 animals were selected for a respiration measurement (see below). Those animals were also used to analyze the carbon content of the different stages. For body carbon content of copepodits and adult copepods, 50 C1 or 30 adult copepods were transferred to tin capsules and dried at 65 °C. Particulate organic carbon was determined by an element analyzer (Thermo Scientific Flash 2000)

Growth and development

The developmental stages of copepods and copepodids were determined for each sampling day. Naupliar stages were not determined separately. Copepodids were discriminated regarding their 6 copepodit stages. To calculate the mean developmental stage of a sample we assigned a value of 6 to all naupliar stages, and scored the six following copepodid stages with 7-12. This clearly leads to a slight overestimation of developmental rates. However, as we only used nauplii hatched within 24h for our experiment, the stage distribution was always very narrow.

Naupliar stage 1-6 = 6, C1=7, C2=8, C3=9, C4=10, C5=11, adult=12

Developmental rate = ∑(NS) / total number of animals in the treatment / days of growth,

where *N* was the number of copepods at certain stage and *S* the assigned stage value (Ismar *et al.*, 2008, Malzahn *et al.*, 2010, Schoo *et al.*, 2013).

Furthermore, based on the average carbon content of the two analyzed stages (as measured for a subset of the animals after harvesting) we used the carbon content to compute the somatic growth rates (d^{-1}). We used the carbon-size relationship of our animals to calculate the carbon content of adult copepods that died before sampling and analyses. Copepods that should grow until adulthood and died before sampling were fed P-limited algae at the temperatures 15 °C, 17 °C, and 20 °C for each food treatment, respectively (Supplementary material). All animals of each experimental vial were pooled. Carbon contents were converted into somatic growth rates per day using the formula $g = [\ln(W_1)-\ln(W_0)]/$ days of growing from C1 sampling to adult sampling, and from egg (assuming *Acartia* sp. eggs have 45.7 pg C egg^{-1} (Kiorboe *et al.*, 1985)) to C1 copepodit stage, where W_0 is the carbon content of egg or C1 stage, and W_1 the carbon content of C1 stage or adult copepods after Boersma *et al.* (2001).

Length measurements

For length measurements, subsamples of copepods were fixed with Lugol's iodine and developmental stages were identified subsequently. Prosome lengths of adults and C1-developmental stages were measured to the nearest µm using a ZEISS Discovery V.8 microscope at 25-fold and 40-fold magnification via photographs and digital software (ZEISS AxioVision 4.8 and AxioCam MRc). Prosome length of only C1 copepodids and adults were measured and standard deviations of the replicates were calculated respectively in each treatment.

Respiration

Respiration as oxygen consumption of copepods was measured with a microsensor oxygen meter (PreSense Precision Sensing, Germany) equipped with oxygen micro-optodes. The copepods were washed in a 75 µm mesh sieve to separate them from phytoplankton and transferred to 2 mL glass vials. Approximately 50 copepodits or 30 adult copepods from each replicate were taken for respiration measurements. A 2-point calibration was done for every temperature treatment. The oxygen consumption was measured for 20 minutes, where the glass vials were filled with 2 mL artificial seawater at the treatment temperature and stored within a water bath to keep the temperature stable. During the measurements all vials were kept in the dark. In parallel, blank samples that consisted only of artificial sea water were measured to correct for potential bacterial respiration. The copepods were adapted to the new environment for five minutes before starting the measurements. Respiration rates were calculated by linear regression of water oxygen concentration over time. The resulting oxygen consumption (µL O_2 C^{-1} h^{-1}) was calculated. Afterwards, the copepods were collected and counted to determine the number of animals in each vial, permitting an accurate calculation of respiration rates per individual. Copepods were then collected for body carbon analysis.

Statistical Analysis

The copepod growth rates, prosome length, and respiration were first tested for normal distribution applying Kolmogorov-Smirnoff tests (p=0.05) in RStudio Integrated development environment for R (Version 0.97.551) Boston, MA, USA (available from http://www.rstudio.org/R), and tested for homogeneity of variances. Subsequently, two factorial ANOVAs using food and temperature as independent factors and prosome length, growth rates, respiration and somatic growth rate as response variables respectively. The two factorial ANOVA models were run for C1 copepodids and adults. Tukey's HSD test was used as the post hoc test in all cases.

Results

Developmental and growth rates

The development of the copepods was significantly affected by temperature, food quality, and the interaction of temperature and P-limitation. Young copepodid stages as well as adult copepods had faster developmental rates in warmer temperatures (Fig. 2.1, Tab 2.2). P-limited and P-pulsed algae significantly decreased the developmental rates of *A. tonsa* (Fig. 2.1). Copepods, which were fed P-limited algae, had the slowest developmental rates in both

samples (C1 or adults), significantly. Animals which were fed algae that were grown on P-limitation and later were pulsed with phosphate, developed significantly slower than copepods reared on P-replete algae but faster than copepods fed P-limited *R. salina*. Copepods fed P-limited algae and reared at 20 °C and should grow until adulthood for sampling, died at developmental stage C3.

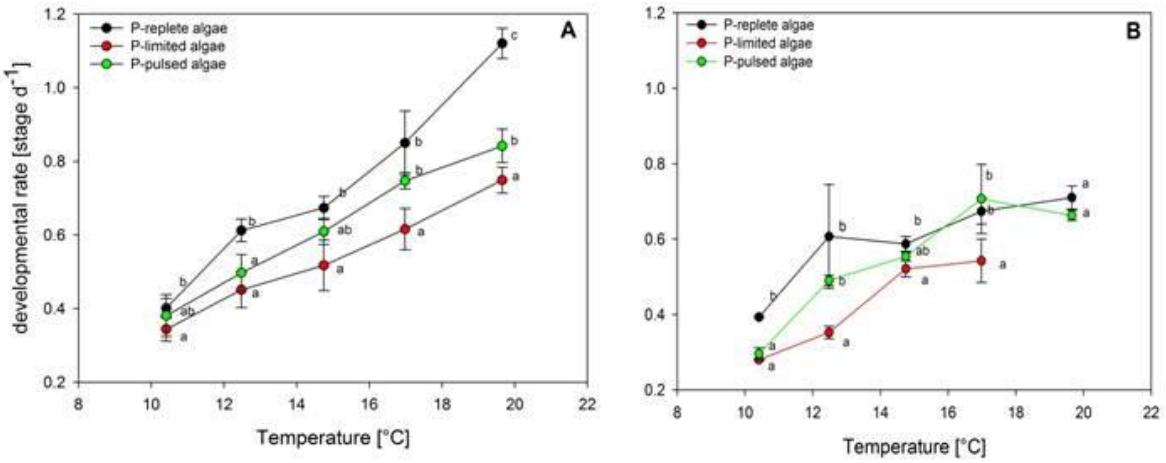


Fig. 2.1 Developmental rate of nauplii to copepodid stage C1 **A)** and from C1 to adult copepods **B)** fed with P-replete (black), P-limited (red), and P-pulsed (green) *R. salina* (mean values of 5 replicates with standard deviation). All adult copepods of P-limited treatment on 20°C fed P-limited *R. salina* on 12°C died. Statistically significant differences between treatments within each temperature (*P<0.05* Tukey's honest significant differences (HSD) test) are indicated by different letters.

Somatic growth of younger stages (nauplii to C1) was less affected by lower P availability than adult copepods (Fig. 2.2). Temperature effects on somatic growth rates are much smaller than on the developmental rates. So, faster developmental rate combined with less affected growth rates yields differently sized organisms, which might explain the different sized animals. The somatic growth rate of copepods that grew from copepodit stage C1 to adult copepods increased significantly with increasing temperatures, but P-limitation of food algae significantly slowed down the somatic growth compared to copepods that were fed nutrient replete algae (Tab. 2.2, Fig. 2.2.). Also, the interaction of food quality and temperature had a significant effect on somatic growth rates. Overall, food quality had significant effects on the developmental rates (Tab. 2.2, Fig 2.1). Copepods that were fed P-replete algae developed significantly faster than animals that were permanently fed P-replete or -pulsed algae. However, copepods had higher developmental rates with P-pulsed algae than those fed P-limited algae. Unfortunately, all copepods fed with P-limited algae and reared at 20 °C died on day 17 of the experiment in the C3 stage.

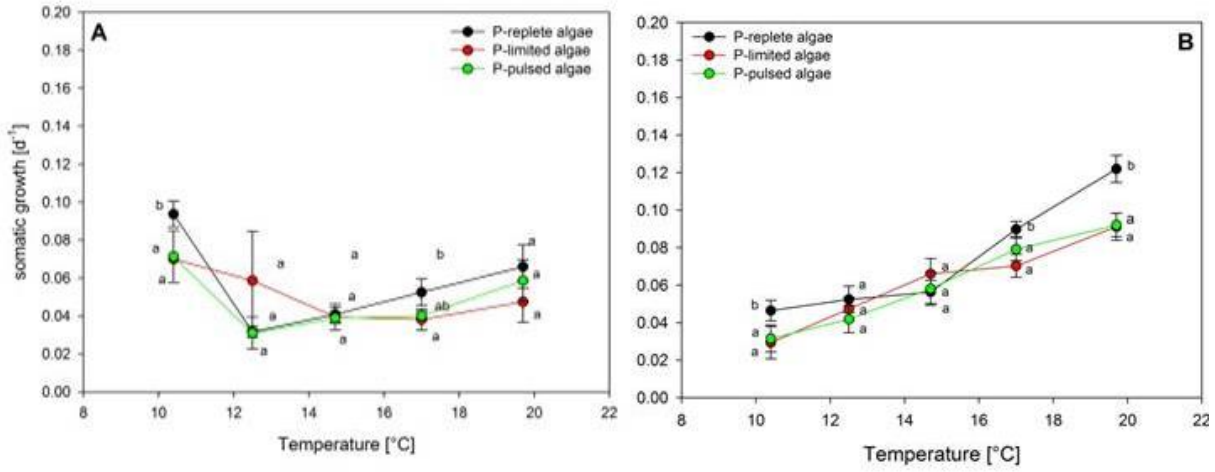


Fig. 2.2 Average somatic growth of *A. tonsa* on different *R. salina* C/P ratios. **A)** egg to copepodid stage C1, and **B)** C1 to adult copepod fed P-replete (black), P-limited (red), and P-pulsed *R. salina* (average values of all 5 replicates with standard deviation). Statistically significant differences (*P<0.05* Tukey's honest significant differences (HSD) test) are indicated by different letters.

Tab. 2.2 Summary of all 2-way ANOVA results with nutrient treatment and temperature as independent factor (values in bold are significant at p < 0.05).

Variable	Factor	Sum Squares	Mean Squares	F-value	p-value	dF
C:P algae	quality	9.679	4.839	2.158	**<0.001**	2
	Residuals	53.825	2.243			24
prosome length C1	Temperature	88438	22109	19.938	**<0.001**	4
	Food	7925	3962	3.573	0.058	2
	Temperature * food	16575	2072	1.868	0.082	7
	Residuals	65425	1109			59
prosome length adult	Temperature	298877	298877	114.2496	**<0.001**	4
	Food	4093	2047	0.7823	0.462	2
	Temperature * food	5504	2752	1.0520	0.356	7
	Residuals	149112	2616			57
Developmental rates nauplii-C1	Temperature	2.253	0.563	257.86	**<0.001**	4
	Food	0.499	0.249	114.18	**<0.001**	2
	Temperature * food	0.177	0.022	10.14	**<0.001**	7
	Residuals	0.125	0.002			57

Developmental rate C1 to Adult	Temperature	2.189	2.189	670.85	**<0.001**	4
	Food	0.492	0.246	75.37	**<0.001**	2
	Temperature * food	0.156	0.078	23.94	**<0.001**	7
	Residuals	0.215	0.003			66
Somatic growth egg to C1	Temperature	0.405	0.101	1093.3	**<0.001**	4
	Food	0.191	0.095	1028.3	**<0.001**	2
	Temperature * food	0.088	0.0126	135.5	**<0.001**	7
	Residuals	0.007	0.000			74
Somatic growth C1 to Adult	Temperature	0.053	0.013	43.22	**<0.001**	4
	Food	0.007	0.004	12.19	**<0.001**	2
	Temperature * food	0.046	0.007	21.24	**<0.001**	7
	Residuals	0.023	0.0003			74
Respiration adult	Temperature	0.052	0.052	24.135	**<0.001**	4
	Food	0.005	0.003	3.155	**<0.05**	2
	Temperature * food	0.015	0.007	3.469	**<0.05**	7
	Residuals	0.086	0.002			74

Body size

Differences in prosome length between copepods reared at five different temperatures were significant in adults and C1 of *Acartia tonsa.* In both developmental stages mean prosome length decreased with increasing temperature (Tab. 2.2, Fig. 2.3). We did not find significant effects of food or interaction of food quality and temperature on mean prosome length (Tab. 2).

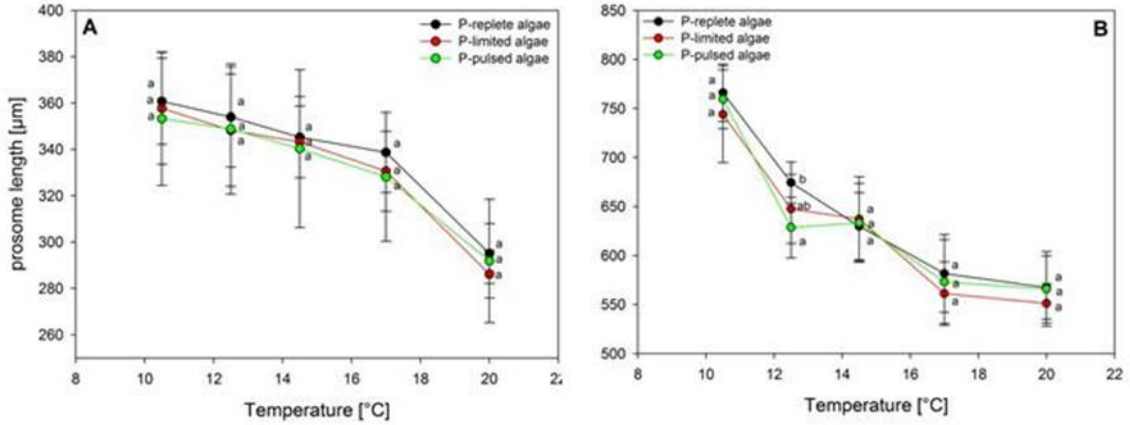


Fig. 2.3 Average values of prosome length of each treatment **A)** copepodid stage C1 and **B)** adult copepods fed with P-replete (black), P-limited (red), P-pulsed (green) *R. salina* (with standard deviation of replicates). Note different scales between C1 and adult copepods. Statistically significant differences (*P<0.05* Tukey's honest significant differences (HSD) test) are indicated by different letters.

Respiration

Adult copepods consumed significantly more O_2 in warmer treatments than copepods in colder treatments (Tab. 2, Fig 2.4). Surplus carbon in food algae led to a significantly higher O_2 consumption compared to copepods, which were fed P-replete algae. C1 copepodids consumed significantly more O_2 at warmer temperatures than copepodids in colder treatments. Copepodids fed P-replete algae at 20 °C had significantly higher O_2 consumption than animals fed P-limited and –pulsed algae.

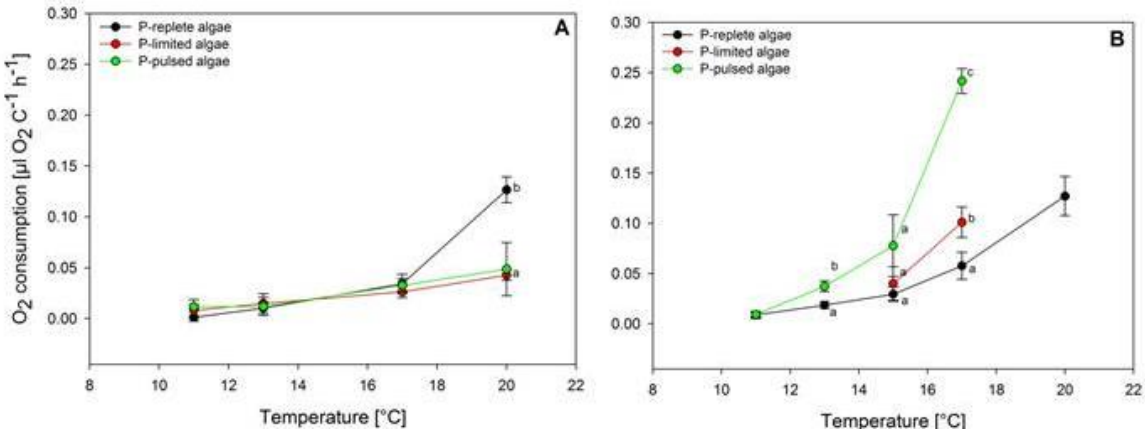


Fig. 2.4 Oxygen consumption rates per dry weight in µg (average values and standard deviation of 5 replicates per treatments. **A)** C1 copepodids and **B)** adult copepods fed P-replete (black), P-limited (red), and P-pulsed (green) *R. salina*. Statistically significant differences (*P<0.05* Tukey's honest significant differences (HSD) test) are indicated by different letters.

Discussion

This study shows that copepod body size decreased at higher temperature in all developmental stages, and increased oxygen consumption rates, somatic- and developmental growth, while P-limitation slowed down somatic growth and developmental rates. Oxygen consumption rates increased with P-limitation.

We suggest that the final body size is influenced indirectly by development- and somatic growth rates due to an increasing metabolism. Developmental rates from larval to mature stages are accelerated more than the increase in individual biomass, or somatic growth, and consequently, animals reach further developmental stages at a smaller size.

Van der Have and de Jong (1996) stated that two processes determine body size-at-stage of an organism: cell differentiation and cell growth. Growth is only an increase in biomass, but growth rate is measured in the dimension of biomass change per unit time irrespective of developmental stage. Differentiation means the diversification of cell types during development and proceeds primarily by cell divisions. Van der Have and de Jong (1996) propose that the invariant number of cells per stage and development defines developmental stage. Temperature effects on growth and development rate of *Acartia* sp. have been experimentally studied and also have been observed in field. Development from egg to adult stage was faster with increasing temperatures under food saturating conditions (Leandro *et al.*, 2006). Leandro *et al.* (2006b) observed that naupliar development stages have higher weight-specific growth rates with warming than copepodits of stages C1-C5.

Our results show that developmental rates and somatic growth both increase with warming. But developmental rates were more affected by increasing temperature, shown in a steeper slope with temperature, compared to the lower response of somatic growth rates to warming. This pattern has also been observed between two allopatric populations of the copepod *A. clausi*, a congeneric of our study species. Forster *et al.* (2011) suggested decoupling growth (increase in mass) and development (passing through life stages) because of different temperature sensitivities of both parameters. Growth depends primarily on the rate of protein biosynthesis which is limited by diffusion of the ribosomal subunits before starting with translation, whereas development depends on enzymatic regulated DNA replication, and cell division (van der Have and de Jong, 1996). Diffusion at higher temperatures may be more rate-limiting to protein synthesis than DNA replication, the temperature coefficient of growth can be expected to be lower than the temperature coefficient of cell differentiation or development with increasing temperature (Forster *et al.*, 2011, Huntley & Lopez, 1992, Van Der Have & De Jong, 1996). Brown *et al.* (2004) stated in their 'Metabolic Theory of Ecology' beyond the intimate relationship of temperature and enzymatic driven processes (metabolism) that maintaining an active metabolism that allows for growth and reproduction increases an organism's energetic demands beyond the levels required for staying alive, i.e. respiration (Claireaux & Lefrancois, 2007). Additionally, increasing temperature leads to increasing respiration rates (e.g. Goss & Bunting 1980). Forster and Hirst (2012) suggested that higher respiration or an increased supply of oxygen for respiratory surfaces have to be adapted at higher temperatures, because higher respiration rates consume more energy. Organisms have to differentially allocate their available energy to physiological maintenance, growth and reproduction forcing them to compromise somatic growth to enable basic physiological processes.

A smaller prosome length thus results because growth rate does not match higher developmental rates due to higher environmental temperatures (van der Have and de Jong, 1996). Additionally, organisms have to adopt mechanisms either to increase the oxygen supply for higher respiration or to reduce the metabolic demand by maturing at smaller size at higher temperatures because organisms have to mitigate any progressive reduction in oxygen limitation in the warm. Therefore, temperature-dependent size changes might reduce energy costs until reaching maturity for reproduction, and aids to uphold population growth. Cell division and differentiation are more rapid, but cellular growth rate remains relatively constant which results smaller prosome size-at defined stage due to smaller average cell size.

Phosphorus is essential for all organisms, because it is needed in RNA, protein synthesis, and the energy carrier ATP (Elser *et al.*, 2000, Vrede *et al.*, 1999). Our experimental results show that somatic growth is depending on P availability. Copepods had slower somatic growth with P-limitation than animals fed with P-replete algae. Malzahn & Boersma (2012) could show that phosphorus supply is strongly coupled to RNA synthesis and growth, and therefore a need of phosphorus could lead to a limitation of RNA and a lower biosynthesis rate. Changes in RNA-to-DNA ratio are suggested to be directly correlated with somatic growth (Clemmesen, 1993, Malzahn & Boersma, 2012). Lower RNA-to-DNA ratio suggests that a lower amount of RNA, and consequently lower protein synthesis, remains for an increase in mass, whereas a higher amount of DNA reflects a larger number of cells. P-limitation might slow down metabolic processes and DNA replication for development. Copepods under P-limitation have less phosphorus to use maintaining basic physiological processes, which results in reduced growth rates. Faster developmental rates due to higher environmental temperatures cannot be supported by phosphorus when copepods grow under P-limitation because higher metabolic rates need a higher amount of phosphorous to cover the costs of the faster physiological processes. Interestingly, we found different dependences on phosphorus in young and more advanced developmental stages. Somatic growth rates of nauplii to C1 copepodit stage were less affected than animals C1 copepodits to adult copepods. Accordingly, animals in our study needed longer for transition from one stage to another under P-limitation, but developed faster with increasing environmental temperature. Carrillo *et al.* (2001) suggested in their study that nauplii may be more affected by phosphorus limitation because they have high growth rates and have to invest most of their resources into growth and moulting, whereas adults have to split their available resources between growth, maintenance, survival and reproduction. Different copepod stages may have different relationships between elemental content and ontogenetic parameters. These relationships may be explained by different life-history strategies in nauplii versus copepodits and adults. Carrillo *et al.* (2001) argued that nauplii have significantly higher nitrogen contents due to the positive relationship with both parameters (body size and growth rate). Additionally, nauplii need more body P for more rapid growth than adults (Elser *et al.*, 1996), which is supported by the found relationship between growth rate and RNA content in copepods (Saiz *et al.*, 1998). High nauplii growth rates require a high ribosomal complement for extensive protein synthesis, whereat copepodits and adults are characterized by a decrease in the specific P content as the organism's ontogenetic development progressed.

P-limitation and consequently a surplus of carbon in food algae unbalances the homeostasis of their C-to-nutrient ratios (Sterner & Elser, 2002). Herbivores aim to maintain homeostasis, by disposing of surplus carbon through respiration (CO_2), and excretion of DOC (Goss &

Bunting, 1980, Schoo *et al.*, 2013). Copepods have to invest more energy to excrete the up taken surplus carbon (Boersma, 2000, Malzahn *et al.*, 2007, Malzahn *et al.*, 2010), in combination with higher temperature, an higher amount of energy is used for higher respiration rates, but also for the temperature-dependent faster development until maturity than at lower temperature and P-replete food.

Interestingly, developmental- and somatic growth rates of copepods reared on P-pulsed food algae, showed an intermediate response between the other treatments. These had lower developmental rates than P-replete fed animals, but showed faster transition from stage to stage than copepods reared at P-limited conditions. Yet, somatic growth rates of P-limited and P-pulsed copepods were significantly different from those in copepods fed with P-replete algae. This indicates that the difference between P-limited and P-replete algae is not only the phosphorus content of the algae food, as the P-content of the pulsed and replete algae is almost identical. As the fatty acid spectrum of P-replete *R. salina* is actually better in terms of highly unsaturated essential fatty acids (Breteler *et al.*, 2005, Malzahn *et al.*, 2007, Malzahn *et al.*, 2010) the factor that is responsible for the rest of the food quality difference between the differently cultured algae remains elusive.

Our study shows that warming and P-limitation affect consumers' development, growth, and body size. We found that that warming increases developmental- and somatic growth rates and increases in C-to-nutrient ratio slow down developmental- and somatic growth rates. P-limitation within food particles lead to higher excretion of surplus carbon that copepods cannot only allocate less to growth and development which means copepods have to grow slower. Our findings show that body size declines with increasing temperature and that this is only driven by temperature and unaffected by food quality. The mismatch between temperature driven faster development and slower somatic growth with P-limitation leads to a longer growth period and reach a smaller size for reproduction. Copepods body size decrease might result because higher metabolic rates increase the demand of phosphorus to cover enhanced growth rates and other physiological processes at higher temperatures. Our results support our first hypothesis that copepods at higher temperature have higher developmental rates and phosphorus demand, and are consequently more affected by P-limitation and high temperature than by no P limitation and high temperature.

Size response to warming is expected to affect ecosystems substantially by modifying overall size-structures of communities, as well as size-dependent biogeochemical rates in organisms and food web dynamics. Malzahn *et al.* (2007) stated that low food quality of autotrophs negatively impacts on the quality of zooplankton, which in turn affects planktivorous fish. This finding may have implications for the predictions of fish populations because quality, individual size, development and abundance of their prey will change with rising temperature and phosphate limitation. Smaller body size at maturity might influence predators, such as fish larvae through the lower energetic value as a food source, consequently insufficiently covering their energetic demands of enhanced metabolism at increased temperature

Conclusions

We showed that warmer water temperatures significantly decrease individual copepod body size, developmental- and somatic growth rates significantly increase, and respiration rates also significantly increased. Copepods that fed P-limited algae had slower developmental and somatic growth rates, and higher respiration rates than copepods that fed on P-replete

algae. Increasing temperature enhances growth but parallel low phosphate availability lead to a mismatch and copepods need longer to reach adulthood. Simultaneous stress of increasing temperature and lower phosphate availability enhances the energy costs of copepods to maintain basic metabolism and increases expenses for reproduction. We could show that phosphorus availability, as a term for food quality, affects physiological conditions of copepods but also that P availability cannot dampen temperature effects on body size. P-limitation has the potential to slow down the development to maturity and might lead to delays in reproduction. Consequently, nutrient limitation due to warming inducing stratification of ocean surface waters could produce long-lasting and cascading effects on secondary production in pelagic systems.

CHAPTER 3

Multi-stressor impacts of ocean warming and acidification on copepod abundance, body size and fatty acid content

Abstract

Concerns about increasing atmospheric CO_2 concentrations and global warming have initiated studying these multi-stressor consequences for marine systems, marine communities, and effects on the individual. We present a mesocosm study assessing how warming and acidification according to predictions for the Baltic Sea, in a two-factorial design, affect copepod body size, abundance, developmental speed and fatty acid composition as a measure of nutritional quality. The experimental setup allowed us to investigate whether the effects of acidification and warming on body size, abundance and fatty acid content of phytoplankton reinforce or counteract each other. The prosome length of the herbivore consumer was significantly reduced with warming, but significantly increased under acidification, and, additionally, there was a significant interaction term between both parameters. The copepod abundance on the final experimental day was significantly lower under warmer conditions. Copepod biomass decreased significantly with warming and increased with acidification, also with a significant antagonistic effect of both parameters indicated by their significant interaction term. Fatty acid composition was significantly affected by warming. The content of saturated fatty acids increased and the essential fatty acids docosahexaenoic acid, docosahexaenoic-to-eicosapentaenoic acid, linolenic acid, and arachidonic acid decreased significantly with higher temperatures. The content of the essential fatty acid ARA decreased significantly with higher CO_2 and showed a significant interaction term of warming and acidification. Our results indicate that in a future ocean scenario with simultaneous warming and acidification, acidification might counteract to some extent the observed temperature effects on copepod prosome length and on copepod abundance and biomass by exerting a fertilizing effect on phytoplankton as a copepod food source. Copepod populations might be more affected by temperature increase than by ocean acidification alone.

Introduction

The concentration of atmospheric carbon dioxide (CO_2) has increased from 280 µatm in pre-industrial times to a present day level of 391 µatm (Le Quéré *et al.* 2013). Over the last 100 years, this had led to changes in global sea surface temperatures (+ 0.74 °C) and ocean carbonate chemistry (Orr *et al.* 2005), which have included ocean acidification by 0.1 pH units (Caldeira and Wickett 2003).

The IPCC reports of the years 2007 and 2014 predict regional temperature increase of 6 °C until the years 2100 respectively 2300. The atmospheric CO_2 is predicted to rise from current values of approximately 390 µatm to 700 µatm for the open ocean at the end of the 21st century. In contrast to the open ocean, where seawater pCO_2 is close to the atmospheric

values, pCO_2 in coastal habitats is more variable (Melzner *et al.* 2013). Shallow temperate estuaries such as Western Baltic Sea, with a strong seasonal vertical stratification due to salinity and temperature gradients, are characterized by higher and fluctuating seawater pCO_2 (Melzner *et al.* 2013, Thomsen *et al.* 2010). Upwelling water masses with high dissolved inorganic carbon content to the surface drastically elevate pCO_2 in the surface layer of Kiel Fjord during summer and autumn. Kiel Fjord surface pCO_2 exceeds present average ocean pCO_2 values during large parts of the year (Thomsen *et al.* 2010). pCO_2 varies between 375 and 2309 µatm (Thomsen *et al.* 2010). These changes in temperature and ocean carbonate chemistry are considered to lead to changes in physiological performance of individual organisms, which will in turn alter biotic interactions, community structure, and ecosystem functioning.

A range of marine biological responses have already been observed in response to ocean warming. Multiple lines of evidence suggest that phytoplankton biomass and productivity (Behrenfeld *et al.* 2006) and mesozooplankton abundances (Edwards and Richardson 2004, Garzke *et al.*, unpublished data) are decreasing, species ranges are shifting (Parmesan and Yohe 2003), body sizes are altered and phenological changes arise (Daufresne *et al.* 2009, Garzke *et al.*, unpublished data). Experimental manipulations simulating predicted future ocean temperatures have suggested that warming will also lead to increased metabolic costs for animals and plants (O'Connor *et al.* 2007), increased consumption rates (Sanford 1999), and changed food-web structures (Petchey *et al.* 1999).

Observed responses of marine organisms to recent ocean acidification are limited, but are expected to become increasingly apparent in the next 50-100 years (Doney *et al.* 2009). At the base of the marine food web, elevated temperatures and acidification lead to different responses of phytoplankton biomass. Higher pCO_2 can have a fertilizing effect and lead to higher algal biomass (Schulz *et al.* 2013). Only a few studies on copepods document significant negative impacts on egg hatching success and naupliar survival at CO_2 concentrations >5000 µatm (Kurihara *et al.* 2004).

An increased availability of CO_2 availability for primary producers may affect their quality as food for herbivores, as increasing carbon availability has the potential to change the stoichiometry of nutrients, which might lead to limitations in essential nutrients. Malzahn *et al.* (2007) showed that nutrient limitation is changing fatty acid (FA) composition in phytoplankton, which directly affects herbivorous consumers. Rossoll *et al.* (2012) found that, with acidification, somatic growth and egg production decreased in an experiment where a single zooplankton species was fed a single algal species, but at the community level increased pCO_2 effects on zooplankton are buffered by food species richness and more complex trophic interactions (Malzahn *et al.* 2010, Rossoll *et al.* 2013). Apart from acidification effects on the chemical composition of the surface water, changes in CO_2 availability will affect primary producers directly; although, this appears to be highly species specific (Gervais and Riebesell 2001; Nielsen *et al.* 2010, Paul *et al.* unpublished data). Temperature and acidification induced changes in stoichiometry by limited nutrient supply and carbon availability are able to alter fatty acid (FA) composition in algae. FA are often critical factors regulating the energy transfer between primary producers and consumers (Brett and Müller-Navarra 1997), because several polyunsatureated FA are essential for mesozooplankton growth and development, cannot be synthesized *de novo* by the animals and have to be acquired through diet. In particular, long-chained essential polyunsaturated FAs (PUFA) such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and

arachidonic acid (ARA) play important roles in growth, development, and reproduction success in heterotrophs (Brett and Müller-Navarra 1997; Rossoll *et al.* 2012).

Copepods are the most important trophic link between primary producers and fish in the pelagic marine food web (Möllmann *et al.* 2005). Taken together, changes in total zooplankton abundance, intra-specific body size decline, and changes in PUFA composition and amount may alter the flow of energy and matter through the food web and thus impact food web structure and community functioning.

Very little is known on the combined effects of predicted warming and ocean acidification on heterotrophic consumers. We here carried out a mesocosm study to test whether warming and acidification in combination cause enhanced responses on mesozooplankton or if the combined stressors can dampen the responses. Resulting phenological, quantitative and qualitative changes in zooplankton life-history, abundance and nutritional composition are assessed.

Materials & Methods

Experimental design

A 24-day mesocosm experiment was conducted using a natural early autumn plankton community (October 19th to November 12th 2012). Unfiltered water from Kiel Fjord containing the natural composition of algae, bacteria and protozoa was used to fill the mesocosms. Additionally, mesozooplankton, mainly consisting of copepods, was added from wild net catches. The target copepod concentration was 10 Ind L^{-1} in order to mimic natural densities (Behrends 1996). The plankton was gently stirred by a propeller for homogeneous mixing of the water column without incurring zooplankton mortality (Sommer *et al.* 2007). Twelve indoor mesocosms, each with a volume of 1400 L, were full-factorial manipulated with two temperature regimes (9 °C, here after called cold, and 15 °C, hereafter called warm) and two CO_2 levels with the target levels of 560 µatm (hereafter called low) and 1400 µatm (hereafter called high) to mimic the extent of warming and acidification predicted for this season and region (IPCC 2007). The experiment commenced three days after filling, when target temperatures and levels of CO_2 had been reached in all treatments. Each mesocosm was covered by a PVC lid containing a sampling port which remained closed between sampling events.

CO_2 manipulation was conducted during the experimental period by a flow of 30-60 L h^{-1} CO_2-enriched air (560 µatm and 1400 µatm CO_2) through the headspace of the mesocosms. The chosen pCO_2 treatment values as 560 µatm (low) and 1400 µatm (high) are within the regional prediction for the high CO_2 environment western Baltic Sea and Kiel Fjord. To balance the natural draw down of CO_2 by phytoplankton production, over the course of the experiment, CO_2 enriched water was added to the high CO_2 mesocosms at three times (October 29th, November 2nd and 9th). For this purpose water taken from the mesocosms was filtered (0.2 µm pore size), CO_2 saturated by bubbling, and again transferred into the mesocosms. The required volumes were calculated on the basis of DIC and alkalinity.

Each treatment was replicated three times. The resulting setup was installed in 4 temperature controlled culture rooms. Light supply and day length were adjusted according to the seasonal patterns expected at this latitude and season (Lewandowska and Sommer

2010). Light was supplied by computer controlled light units (GHL Groß Hard- und Softwarelösungen, Lampunit HL3700 and ProfiluxII). Above each of the mesocosms one unit each consisting of 5 HIBay-LED spotlights (purpose build item of Econlux, each 100 W) was installed. Daily irradiance patterns were constant over the experiment and computer controlled (GHL, Prometheus). The light-dark cycle was 11h50 min: 12h10 min. Light supply and day length were aligned to the seasonal light patterns calculated in the astronomic model of Brock (1981). It conformed to 50% of solar irradiance of an approximated cloudless 21st September. Daily maximum light intensity in the middle of the water column was 252.32 µmol m^{-2} s^{-1}.

Sampling and measurements

Total dissolved inorganic carbon (DIC) samples (10 mL) were filled into glass vials (Resteck, Germany) using a peristaltic pump (flow rate 6 mL min^{-1}) and an intake tube contained a single syringe filter (0.2 µm, Sartorius). Filtered samples were poisoned with saturated $HgCl_2$ solution (20 µL), crimped with a headspace of less than 1% and stored in the dark at 4 °C. DIC was measured following Hansen *et al.* 2013 using a SRI-8610C (Torrence, USA) gas chromatograph. For total alkalinity (TA) 25 mL samples were filtered (Whatman GF/F filter 0.2 µm) and titrated at 20 °C with 0.05M HCl-solution (Dickson 1981, Dickson *et al.* 2003) in an automated titration device (Metrohm Swiss mode). The remaining carbonate parameter pCO_2 was calculated using CO2SYS (Pierrot *et al.* 2006) and the constants supplied by Hansson (1973) and Mehrbach *et al.* (1973), that were refitted by Dickson and Millero (1987). Water temperature, salinity and pH were measured daily. Samples for phyto- and microzooplankton biomass variables, chlorophyll a, and phyto-, microzooplankton carbon (microscopy) were taken three times per week (Monday, Wednesday, and Friday). Zooplankton was sampled weekly by three vertical net haul, with an hand-held plankton net (64 µm mesh size and 12 cm diameter). Each net haul sampled a volume of 5.1 L. Zoo- and phytoplankton samples were fixed with Lugol's iodine and microzooplankton samples were fixed with acid Lugol's iodine. The total zooplankton catch was divided in a sample divider, so that ¼ of the total catch volume was counted and identified, copepod developmental stages and sexes could be distinguished accurately. The prosome length constancy between molts enables a clear assignment of size to a given stage. All copepods were identified to genus level by using a ZEISS Discovery V.8 microscope with the magnification between 25x and 40x and, whenever possible, developmental stage was recorded.
Prosome length of identified copepods was measured digitally via photographs and digital software (ZEISS AxioVision 4.8 and AxioCam MRc) with a precision to the nearest µm. Means were calculated stage-specifically for copepods of each genus found in each mesocosm. Biomass was calculated according to Gismervik *et al.* (2002). The mean developmental index was calculated after a modified formula used by Villegas and Kanazawa (1979):

$$DI = \sum (NS)/N_{tot}$$

Where N = number of copepods at certain stage, S = assigned stage value, N_{tot} = total number of copepods staged. Stages were scored as:

C1 = 1, C2 = 2, C3 = 3, C4 = 4, C5 = 5, Adult = 6

To analyze copepod total fatty acid content and fatty acid composition tin cups with 30 adult female *Paracalanus* sp. individuals were pooled and extracted in chloroform / dichlormethane / methanol (1:1:1 v/v/v) following Arndt and Sommer (2014). Prior to extraction two internal standards, heneicosanoic acid (C21) and FAME mix (C19) were added. Methyl esters were prepared by esterification with toluene and H_2SO_4 (1%) in methanol heated up to 50 °C for 12 h. After extraction with n-hexane the fatty acid methyl esters were analyzed with a gas chromatograph (Thermo Scientific Trace GC Ultra with autosampler AS 3000). Peaks were identified by comparison with standard mixtures.

Statistical analysis

The copepod prosome length, abundance, and Shannon Wiener Diversity Index were firstly tested for normal distribution applying a Kolmogorov-Smirnov test for temperature and CO_2 effects (α=0.05) in SigmaPlot version 11.0 Systat Software, Inc., San Jose California USA. Subsequently, 2-way ANOVAs of the last experimental day data were formulated in RStudio, Version 0.97.551, RStudio Inc., to investigate temperature and CO_2 effects on prosome length, copepodide abundance, and Shannon Wiener diversity index. The 2-way ANOVA models for prosome length and abundance were run for all occurring taxa of copepodits and adults and as well the predominant taxon *Paracalanus* sp. Temperature and CO_2 treatments were set as categorical explanatory variables, in separate ANOVAs with prosome length, abundance, and Shannon Wiener Diversity as respective continuous response variables. Size analyses were conducted with the response variable prosome length for different zooplankton groups: (1) for all adult copepods of all occurring taxon, (2) for all developmental stages of the most abundance taxon *Paracalanus* sp. separately. Abundance analyses were conducted for the last experimental day by using 2-way ANOVA and across the whole experimental duration by deploying a repeated measure ANOVA. Here, temperature and CO_2 were set as categorical variables, copepodid abundance as a continuous response variable and day of experiment as repeated measure. The Shannon Wiener diversity Index was deployed to assess diversity changes between the different treatments.

Fatty acid analyses were conducted with adult female *Paracalanus* sp. of the last experimental day by using 2-way ANOVAs with the response variable for the respective FA ratio. Temperature and CO_2 treatments were set as categorical explanatory variable and FA ratio as continuous variable.

Tukey's honest significant difference test was used as the post hoc test for all 2-way ANOVAs and a Bonferroni-corrected pairwise t-test for the repeated measure ANOVA. To test homogeneity of variance, Fligner-Killeen tests were applied in all cases.

Results

Body Size

The mean prosome length of adult copepods at the final day was significantly smaller at higher temperatures (602.83 µm ± 219.36 µm) than at lower temperatures (674.92 µm ± 189.03 µm, Tab. 3.1, Fig. 3.1). This temperature-driven difference was also found in all species-specifically calculated mean prosome lengths (Tab. 3.1, Fig. 3.1). The mean prosome length, also, was significantly bigger at high CO_2 (638.69 µm ± 204.42 µm, Fig. 3.1) compared to mean prosome length of adult copepods at low CO_2 (656.04 µm ± 203.95 µm, Tab. 3.1, Fig. 3.1). Adult prosome length results showed a compensatory effect of both factors, presented in the significant interaction effect of both parameters (Tab. 3.1, Fig. 3.1).

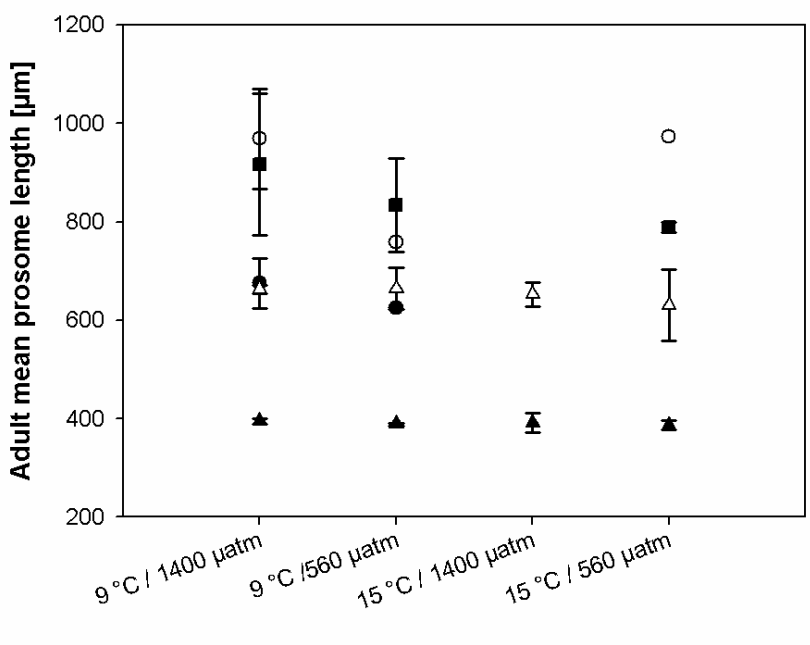


Fig. 3.1 Mean prosome length of adult copepods of last experimental day with SD (closed circle *Acartia* sp., open circle *Centropages* sp., closed triangle *Oithon*a sp., open triangle *Paracalanus* sp., closed square *Pseudocalanus* sp.).

Paracalanus sp. prosome length was significantly smaller at higher temperatures (467.66 µm ± 186.64, Tab. 3.1, Fig. 3.2) when compared to mean prosome length at lower temperatures (525.19 µm ± 122.02 µm). Mean prosome length of *Paracalanus* sp. at high CO_2 (487.22 µm ± 138.19 µm) was not significantly different from mean prosome length at low CO_2 (500.10 µm ± 127.02 µm). The interaction of temperature and CO_2 did not significantly affect mean prosome length of *Paracalanus* sp. developmental stages (Tab. 3.1, Fig. 3.2,).

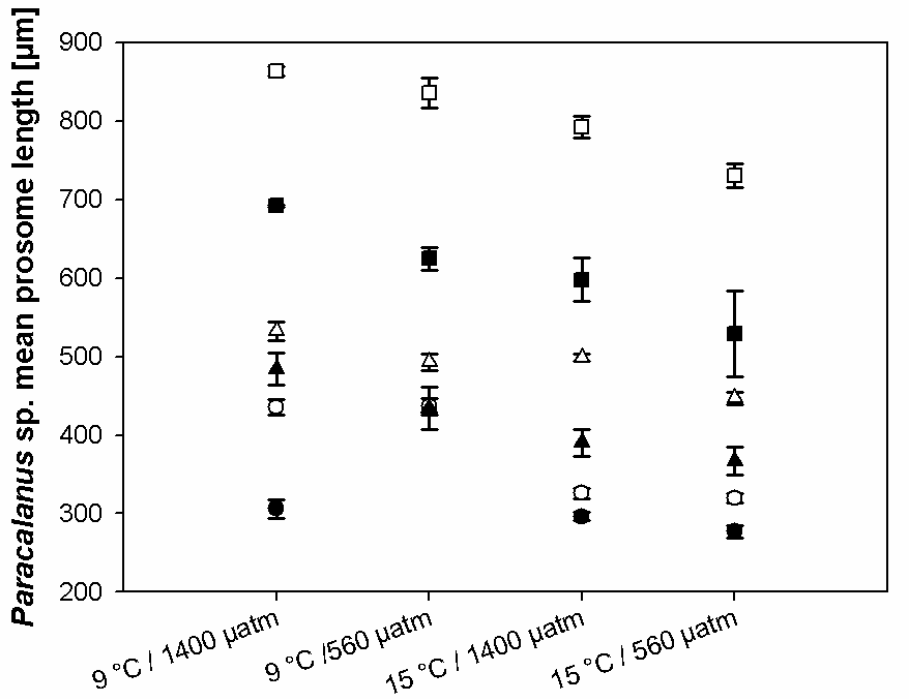


Fig. 3.2 Mean prosome length of al developmental stages of *Paracalanus* sp. (average values with standard deviation; closed circle copepodid stage C1, open circle C2, closed triangle C3, open triangle C4, closed square C5, open square adult).

Tab. 3.1 Results of 2-way ANOVA explaining the effects of temperature and CO_2 on adult copepod prosome length, prosome length of all developmental stages of *Paracalanus* sp., total, nauplii, copepodid, and adult abundance, and adult biomass of the last experimental day. Temporal results of repeated measurement ANOVA with mesocosm ID as repeated measurement explaining the effects of temperature and CO_2 on total, nauplii and copepodid abundance (values in bold are significant at $p < 0.05$).

		Contributing factors			Whole model			
Variable	Factor	dF	F	p-value	dF	R^2	F	p-value
Size adults	Temperature	1	34.59	**<0.01**	18	0.84	28.14	**<0.01**
	CO_2	1	9.96	**0.01**				
	Temperature x CO_2	1	5.59	**0.03**				
	Temperature x species	3	2.65	0.08				
	CO_2 x species	4	4.68	**<0.01**				
	Temperature x CO_2 x species	1	0.27	0.61				
	Residuals	18						

***Paracalanus* sp. stages size**	Temperature	1	24.34	**<0.01**	32	0.89	21.03	**<0.05**
	CO_2	1	0.01	0.93				
	Temp x CO_2	1	0.72	0.40				
	Temperature x CO_2 x stage	1	0.11	0.74				
	Residuals	32						
Total abundance final day	Temperature	1	0.32	**<0.01**	8	0.82	0.21	**<0.05**
	CO_2	1	4.97	0.06				
	Temperature x CO_2	1	0.24	**<0.05**				
	Residuals	8						
Abundance of copepods final day	Temperature	1	0.39	**<0.05**	8	0.91	0.34	**<0.01**
	CO_2	1	0.72	**<0.05**				
	Temperature x CO_2	1	5.02	0.99				
	Residuals	8						
Abundance of nauplii final day	Temperature	1	0.34	**<0.05**	8	0.86	0.28	**<0.05**
	CO_2	1	7.15	0.09				
	Temperature x CO_2	1	0.62	**<0.05**				
	Residuals	8						
Abundance of adult copepods final day	Temperature	1	0.32	0.59	8	0.81	0.27	**<0.01**
	CO_2	1	4.98	0.06				
	Temperature x CO_2	1	0.23	0.64				
	Residuals	8						

Total copepodit biomass last day	Temperature	1	0.55	0.43	8	0.85	0.47	**<0.01**
	CO_2	1	4.41	**<0.01**				
	Temperature x CO_2	1	0.14	**<0.05**				
	Residuals	8						
Total abundance over time	Temperature	1	3.61	0.07	35	0.87	3.02	**<0.05**
	CO_2	1	5.05	**<0.05**				
	Temperature X CO_2	1	1.38	0.25				
	Residuals	35						
Copepodid abundance over time	Temperature	1	2.54	0.12	35	0.89	4.76	**<0.05**
	CO_2	1	5.39	**<0.05**				
	Temperature X CO_2	1	1.84	0.18				
	Residuals	35						
Nauplii abundance over time	Temperature	1	0.28	0.59	35	0.73	**1.03**	**0.05**
	CO_2	1	1.57	0.22				
	Temperature X CO_2	1	1.35	0.25				
	Residuals	35						
Adult copepodit biomass last day	Temperature	1	0.55	0.48	8	0.90	**3.12**	**0.05**
	CO_2	1	4.41	0.07				
	Temperature x CO_2	1	0.15	0.71				
	Residuals	8						

Abundance and Biomass

Total abundance (copepods and nauplii) at the last experimental day was significantly lower at higher temperatures (53.37 Ind L^{-1} ± 11.52) than at lower temperatures (105.82 Ind L^{-1} ± 42.68, Fig. 3.3A, Tab. 3.1). Total abundance was not significantly higher at high CO_2 (94.13 Ind L^{-1} ± 59.21) than at low CO_2 (65.07 Ind L^{-1} ± 14.96, Fig. 3.3C, Tab. 3.1).

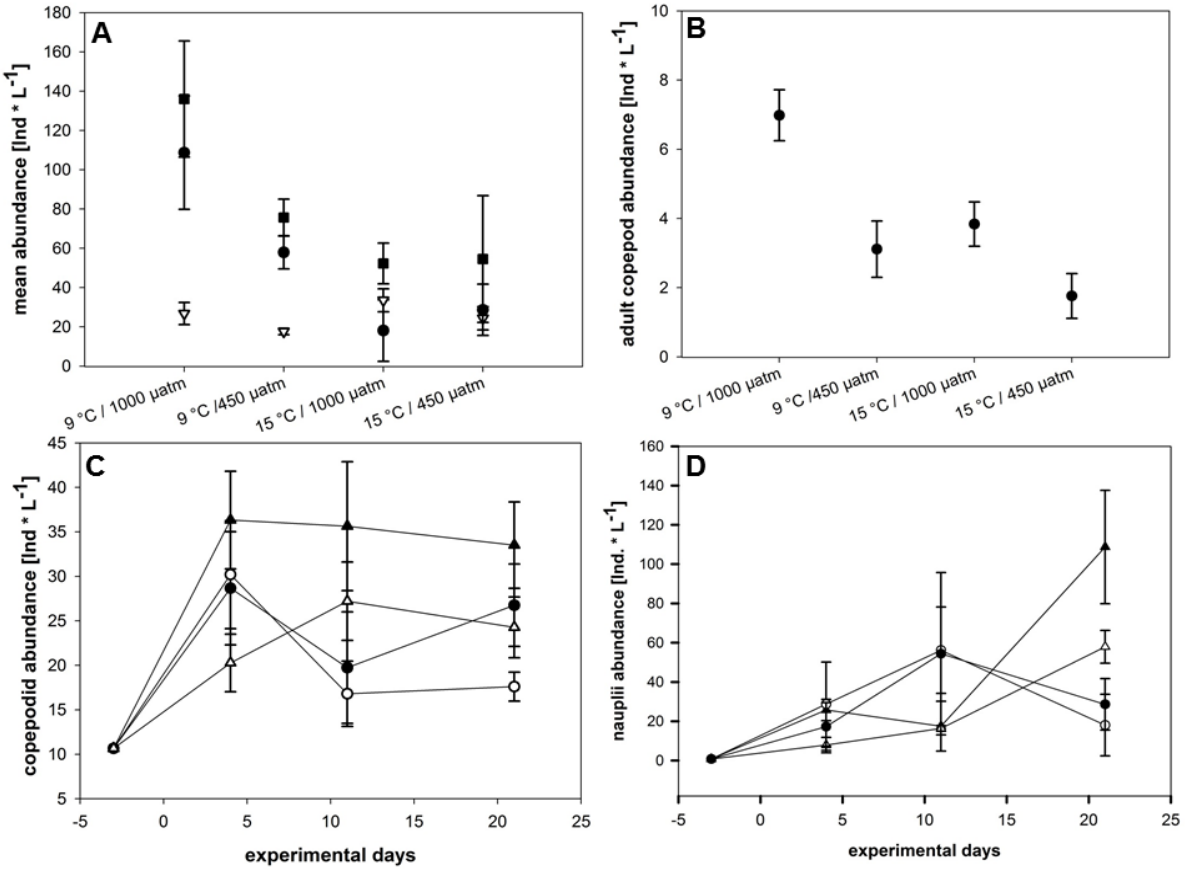


Fig. 3.3 Copepod abundance data (average values with standard deviation) **A)** abundances of nauplii, copepodits, and total of the last experimental day (closed squares total zooplankton abundance, closed circles nauplii abundance, open triangles copepodid abundance) **B)** adult copepod abundance of the last experimental day; **C)** copepodid abundance over time (closed triangles 9 °C and 1000 µatm, open triangles 9 °C and 450 µatm, closed circles 15 °C and 1000 µatm, open circles 15 °C and 450 µatm); **D)** nauplii abundance over time (closed triangles 9 °C and 1000 µatm, open triangles 9 °C and 450 µatm, closed circles 15 °C and 1000 µatm, open circles 15 °C and 450 µatm).

Total copepod abundance results indicate an antagonistic effect of temperature and pCO_2, depicted in the significant interaction effect of both parameters (Tab. 3.1). Copepodid plus adult abundance on the last experimental day, was significantly higher at higher temperatures (28.94 Ind L^{-1} ± 6.47, Fig. 3.3A, Tab. 3.1) when compared to copepodid abundance at lower temperatures (22.47 Ind L^{-1} ± 6.47, Tab. 3.1). Copepodid plus adult abundance at high CO_2 was significantly higher (30.13 Ind L^{-1} ± 4.78, Fig. 3.3A, Tab. 3.1) compared to abundance at low CO_2 (20.98 Ind L^{-1} ± 4.78, Tab. 1) on the last experimental day. Adult copepods tended only to be more abundant at high CO_2 (5.41 Ind L^{-1} ± 2.22,

Tab. 3.1) when compared to adult abundance at low CO_2 (2.44 Ind L^{-1} ± 0.96) and less abundant at higher temperatures (2.79 Ind L^{-1} ± 1.47) when compared with abundances of adult copepods at lower temperatures (5.05 Ind L^{-1} ± 2.74, Fig. 3.3A, Fig. 3.1). Nauplii abundance at the last experimental day was significantly lower at higher temperatures (23.33 Ind L^{-1} ± 7.48, Fig. 3.3A, Tab. 3.1) compared to nauplii abundance at lower temperature (83.29 ± 35.95 Ind L^{-1}, Fig. 3.3A, Tab. 3.1). Nauplii abundance increased at high CO_2 (63.38 Ind L^{-1} ± 28.87, Fig. 3.3A, Tab. 3.1) when compared to low CO_2 treatments (43.24 Ind L^{-1} ± 15.65, Fig. 3.3A, Tab. 3.1). Nauplii abundance showed a compensatory effect of both factors, depicted in the significant interaction effect of both parameters (Fig. 3.3A, Tab. 3.1). When averaged over the entire time of the experiment, copepod abundance was significantly higher at high CO_2, whereas temperature had no significant effect on copepod abundance over time (Fig. 3.3C, Tab. 3.1).

The mean developmental index (MDI) was significantly different between higher and lower temperatures (Tab. 3.4). The MDI of lower temperature treatments was 1.77, which means that the copepods on average were between copepodid stage C1 and C2 (Tab. 3.4). At higher temperature the MDI was between copepodid stage C2 and C3 (Tab. 3.4). The differences of average developmental stage indicate a phonological shift between temperature treatments. This can be observed in the abundance distribution of the different developmental stages (nauplii, copepodits adults) of the last experimental day (Fig. 3.3A).

Tab. 3.2 Mean developmental index of last experimental day

treatment	MDI
Cold / high	1.77
Cold / low	1.87
Hot / high	2.87
Hot / low	2.50

Copepodid plus adult biomass was significantly higher at high CO_2 (70.03 µg C L^{-1} ± 32.72) compared to low CO_2 treatments (37.10 µg C L^{-1} ± 18.14, Tab. 3.1, Tab. 3.3). Temperature had no significant effect on copepodid plus adult biomass (Tab. 3.1). The biomass results showed a compensatory effect of both factors, depicted in the significant effect of temperature and pCO_2 (Tab. 3.1). Biomass of adult copepods was not significantly affected by temperature, CO_2 or the interaction of both (Tab. 3.1).

Tab. 3.3 Extend of differences in mean prosome length, biomass and abundance changes of adult copepods between treatments

		Extend warm to cold	Extent high to low
Size	Adult *Paracalanus* sp.	21.29 µm	9.82 µm
	C5 *Paracalanus* sp.	20.26 µm	35.79 µm
	C4 *Paracalanus* sp.	14.58 µm	20.67 µm
Biomass adults		-35.96 µg C * L^{-1}	2.97 µg C * L^{-1}
Abundance adults		-2.25 Ind * L^{-1}	2.97 Ind * L^{-1}

Fatty acid composition

The total amount of fatty acids per adult female individuals of *Paracalanus* sp. did not change between the different treatments (Tab. 3.4, Fig. 3.4A). The ratio of saturated fatty acid-to-total fatty acids (SFA/TFA) was significantly higher at the higher temperature and the interaction of temperature and CO_2 did significantly affect SFA/TFA (Fig. 3.4A, Tab. 3.4).

Even though total PUFAs were not statistically affected by temperature and/or CO_2, single polyunsaturated fatty acids were affected. The DHA/TFA ratio (docosahexaenoic acid (22:6(n-3)) significantly decreased with rising temperature (Tab. 3.2, Fig. 3.4D). EPA (eicosapentaenoic acid (20:5(n-3)) was not affected by temperature and CO_2 (Tab. 3.2, Fig. 3.4E). The arachidonic acid (ARA (20:4(n-4)) on the other hand, significantly increased with higher temperature (Tab. 3.2, Fig. 3.4F) and higher CO_2 (Tab. 3.2, Fig. 3.4F), as well as showing a synergistic effect of both factors, depicted in the significant interaction effect of both parameters on ARA content (Tab. 3.2, Fig. 3.4F). The ratio of DHA/EPA ratio significantly increased with lower temperatures (Tab. 3.2, Fig. 3.4G). Linolenic acid (18:3(n-3)) decreased significantly with increasing environmental temperature (Tab. 3.2).

Tab. 3.4 Results of 2-way ANOVA explaining the effects of temperature and CO_2 on fatty acids (values in bold are significant at $p < 0.05$).

		Contributing factors			Whole model			
Variable	Factor	dF	F	p-value	dF	R^2	F	p-value
PUFA:TFA	Temperature	1	2.38	0.17	7	0.87	1.97	**<0.05**
	CO_2	1	0.66	0.44				
	Temperature x CO_2	1	0.01	0.92				
	Residuals	7						
HUFA:TFA	Temperature	1	0.56	0.48	7	0.89	0.47	**<0.05**
	CO_2	1	0.02	0.91				
	Temperature x CO_2	1	0.07	0.79				
	Residuals	7						

SFA:TFA	Temperature	1	25.37	**<0.01**	7	0.86	21.54	**<0.05**
	CO_2	1	0.18	0.68				
	Temperature x CO_2	1	7.53	**<0.05**				
	Residuals	7						
MUFA:TFA	Temperature	1	5.55	0.05	7	0.85	4.30	**<0.05**
	CO_2	1	1.90	0.21				
	Temperature x CO_2	1	5.07	0.06				
	Residuals	7						
DHA:TFA	Temperature	1	5.78	**<0.05**	7	0.84	5.02	**<0.05**
	CO_2	1	0.06	0.82				
	Temperature x CO_2	1	0.31	0.59				
	Residuals	7						
EPA:TFA	Temperature	1	0.69	0.43	7	0.92	0.45	**<0.05**
	CO_2	1	0.24	0.64				
	Temperature x CO_2	1	0.10	0.76				
	Residuals	7						
ARA:TFA	Temperature	1	19.35	**<0.01**	7	0.89	17.46	**<0.05**
	CO_2	1	7.29	**<0.05**				
	Temperature x CO_2	1	7.06	**<0.05**				
	Residuals	7						
DHA:EPA	Temperature	1	23.61	**0.001**	8	0.84	21.03	**<0.05**
	CO_2	1	0.30	0.59				
	Temperature x CO_2	1	1.39	0.27				
	Residuals	8						
Linolenic acid :TFA	Temperature	1	7.61	**<0.05**	7	0.82	6.32	**<0.05**
	CO_2	1	0.01	0.97				
	Temperature x CO_2	1	0.31	0.59				
	Residuals	7						
Linoleic acid:TFA	Temperature	1	0.09	0.78	7	0.73	2.10	**<0.05**
	CO_2	1	2.82	0.14				
	Temperature x CO_2	1	1.45	0.27				
	Residuals	7						

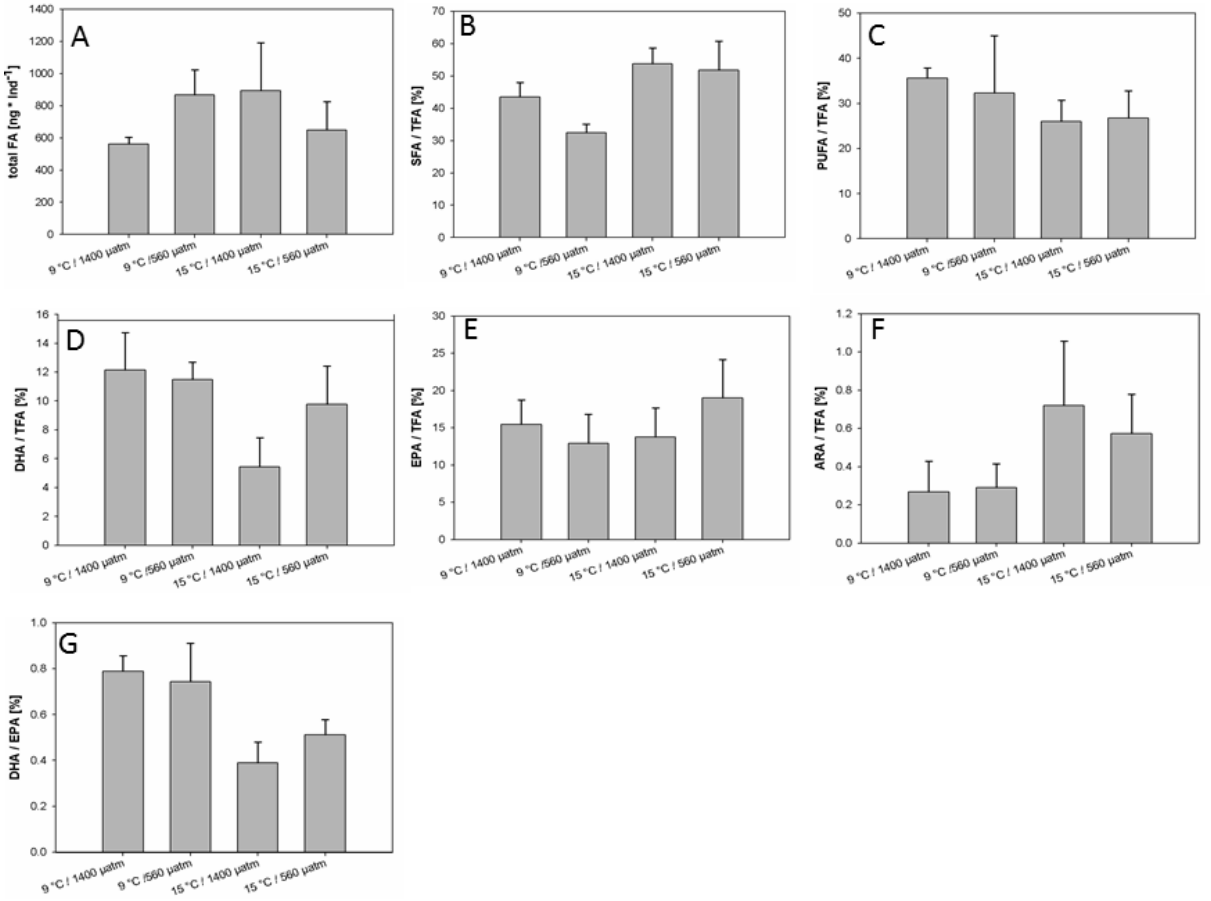


Fig. 3.4 Fatty acid changes between treatments of female adult *Paracalanus* sp. (means with SD **A)** total fatty acid (TFA) content; **B)** SFA-to-TFA ratio; **C)** PUFA-to-TFA ratio; **D)** DHA-to-TFA ratio; **E)** EPA-to-TFA ratio; **F)** ARA-to-TFA ratio; **G)** DHA-to-EPA ratio.

Discussion

This study shows that copepod body size decreased at higher temperatures in all developmental stages, and increased at high pCO_2. We found that warming and acidification have counteractive effects on copepod body size, with a compensation of pCO_2 effect on temperature-driven size changes These results are in line with the Temperature-Size-Rule of Atkinson (1994) and experimental results of Daufresne *et al.* (2009) and Garzke *et al.* (unpublished data). Temperature-dependent reduction of body size in adult as well as in younger developmental stages of copepods could be a result of two factors (1) due to the increasing metabolic demands with increasing temperature (Brown *et al.* 2004; O'Connor 2009), and (2) that developmental and somatic growth rates are differentially temperature sensitive (Forster *et al.* 2011).

Our results suggest that ongoing ocean acidification has weaker impacts on our investigated copepod body size than ocean warming. Havenhand (2012) suggested that the ecologically most important groups of Baltic Sea ecosystems seem to be more or less well adapted to future acidification, whereas zooplankton seems more vulnerable to higher temperatures. Indirect positive CO_2 effects on copepod size, abundance and biomass via affecting food availability are indicated from our results. Phyto- and microzooplankton biomass data, used

as a proxy for available food biomass in our experiment, did show that acidification acts as a fertilizer for phytoplankton but that warm temperature treatments had lower phytoplankton biomass, which was also experimentally shown in other studies (Kim *et al.* 2006; Sommer *et al.* 2012, Paul *et al.* unpublished data, Sommer *et al.* unpublished data).

We observed increased total copepod abundance with warming but lower copepodid abundances. High pCO_2 treatments had higher copepod abundances of nauplii, copepodids, and adults in total but also each category alone. Simultaneously, the stage-composition shifted from C1-C2 dominated to a C2-C3 dominated copepod population structure under increasing temperatures but no differences in stage-composition between high and low pCO_2 treatments were detected. This can be explained by faster maturation of copepods at higher temperatures. At higher temperatures, copepods develop faster to the reproductive stage and can consequently reproduce earlier (Kordas *et al.* 2011). Higher daily mortality (Klein Breteler and Schogt 1994) and lower stage-specific survival rate (Garzke *et al.*, unpublished data) at higher may have resulted in the lower abundance of copepods observed in our samples. Rossoll *et al.* (2013) found higher egg production rates at high pCO_2 levels and concluded that this is most likely a response of adult females to a higher food biomass. Consequently, Rossoll *et al.* (2013) findings provide an experimental explanation for our results of higher copepod abundances at high pCO_2 treatments, where also phytoplankton biomass was higher.

Fatty acid composition of adult *Paracalanus* sp. was generally more affected by temperature change rather than by change of CO_2. SFA-to-TFA ratios increased with warming, as well as individual essential PUFAs, ARA/TFA, linolenic acid/TFA, DHA/EPA contents; only DHA/TFA ratios decreased with warming. The temperature effects on SFA-to-TFA ratios and high pCO_2 are consistent with observations of Rossoll *et al.* (2012). The increase of the SFA proportion can be explained by more SFA incorporated into the cell membranes to maintain viscosity at higher temperatures but also to counteract the decreasing internal cell-pH with acidification (Lane and Burris 1981). DHA, EPA and ARA are important FAs for promoting growth and reproduction of consumers (Jonasdottir *et al.* 2009, Lee *et al.* 1971). Additionally, results of experimental studies showed that decreasing DHA, EPA, and ARA values within the algal food source lower daily egg production rates of female adults (Müller-Navarra *et al.* 2000, Rossoll *et al.* 2012). We did not measure daily egg production, but nauplii abundances of the last experimental day were significantly lower at higher temperatures and at high pCO_2. Nauplii of the last experimental day had hatched during the final stages of the experiment in the different treatments and reflect treatment-dependent egg production rates. We suggest that enhanced developmental rates at higher temperatures increase the need of essential FA for building reproductive tissue and egg production. If food sources have lower amounts of these essential PUFAs, reproductive success is reduced, which was the case during the present study. Since, our study did not involve fatty acid and stoichiometry analyses of phytoplankton we cannot pinpoint a change in both profiles of algae, only for copepods. However, since the measured PUFAs constitute essential fatty acids, they were necessarily food-derived, and it is known from single species studies that algae under high pCO_2 and higher temperatures have lower quality as food source for copepods (Rossoll *et al.* 2012, Schoo *et al.* 2013).

We suggest that a temperature increase, within the regional IPCC predictions until the year 2100, will mainly decrease individual body size, copepod abundance and changes FA composition. Further we suggest that acidification effects, within the IPCC predictions, can have a positive effect on copepod size and abundance by promoting phytoplankton growth

as their main food source, and higher food biomass availability may to some extent supplement temperature-induced higher energy expenses. Yet, negative effects of increasing CO_2-levels may synergistically with warming impede the nutritional quality of copepods for fish. Copepods at higher temperature and low pCO_2 might be food limited and were less able to cover their enhanced metabolism.

Our findings can have implications for higher trophic levels in marine food webs, which call for experimental tests. Smaller copepod prosome lengths and lower abundances at higher temperatures could reduce the matter and energy transfer through the food web, affecting higher trophic levels such as fish larvae (Bochdansky *et al.* 2005), which themselves under warming conditions will have higher metabolic rates and an increasing demand of energy (Brown *et al.* 2004). Changes of the fatty acid composition, especially within the essential PUFAs, might have further consequences for fish development. The observed ratio changes of the essential FAs DHA, ARA, EPA and linolenic acid to the total FA content with warming and acidification has the potential to affect the successful rearing of marine fish where the ratio and the amount play an important role (Sargent *et al.* 1997). In conclusion, our results show that acidification can incompletely counteract warming effects on copepod size, can have differential interactions with warming on copepod abundance, enhancing it by increasing food quantity, while synergistically decreasing food quality components with warming. The directionality of predicted food web impacts of increasing CO_2 under simultaneously increasing temperatures thus depends on whether respective higher-level consumers are food-quantity or quality limited, and need to be tested case-specifically.

CHAPTER 4

Temperature-driven declines in plankton biomass increase mass-specific ecosystem fluxes: higher trophic levels compensate for temperature driven metabolic changes

Abstract

Concerns about global warming have motivated studying the consequences of higher temperatures on ecosystem and individual metabolic fluxes. Despite numerous warming experiments, projections for how warming affects multi-trophic systems remain limited by insufficient understanding of how ecosystem fluxes and species interactions change over broad ranges of temperature. We present a multi-generational mesocosm study assessing how higher temperatures affect and the structure and ecosystem function of the community. The experimental setup allowed us to test the hypothesis that food chain length and trophic level modify the ecosystem-level metabolic effects of temperature. Additionally, we investigated if temperature related responses on phyto- and zooplankton biomass are able to increase mass-specific ecosystem metabolic rates. We found that total net primary production and respiration increased nonlinearly with temperatures but also increased with food chain length. Mass-specific metabolic rates of net primary production and respiration were significantly higher at higher temperatures and increased with food chain length. Phytoplankton and zooplankton biomass decreased continuously with increasing temperature, and body size in the primary grazer species (*Daphnia* sp.) declined at higher temperatures. Warming significantly increased herbivore-to-autotroph biomass ratio, and predation changed the herbivore species composition from larger *Daphnia* sp. dominated to smaller copepod dominated zooplankton composition. Our results indicate that future warmer aquatic ecosystems are more affected at higher trophic levels, because the food web structure is changing to phytoplankton and zooplankton biomass with smaller sized individuals, which have higher metabolic rates.

Introduction

Food webs integrate physiological responses and interactions between organisms across trophic levels. When there are systematic differences in responses to climate factors, such as temperature, among key functional groups in food webs, food web structure and function can vary with warming (Gilbert *et al.* 2014, Norberg & DeAngelis 1997, Vasseur & McCann 2005). Such asymmetric physiological responses to warming have the potential to alter the 'metabolic balance' of an ecosystem, thus linking individual responses to temperature to ecosystem function (Allen *et al.* 2005, Lopez-Urrutia *et al.* 2006). Metabolic balance is defined as the rate of carbon fixation, by photosynthesis, and release by respiration, and determines if an ecosystem acts as a source or sink for atmospheric CO_2 (Woodwell *et al.* 1998). The 'Metabolic Theory of Ecology' (MTE) postulated by Brown *et al.* (2004) describes fundamental biochemical constraints of temperature on enzyme kinetics to determine not only whole-organism metabolic rate, but also broad ecological patterns of population

dynamics, carbon size and also community and individual size changes (Allen *et al.* 2005, Atkinson 1995, Brown *et al.* 2004). This theory therefore provides the testable hypothesis that there is a general and predictable mechanistic link between individual-environment interactions and larger scale ecological patterns.

Yvon-Durocher *et al.* (2012) found that respiration rates in lakes, estuaries, open-ocean and forested terrestrial ecosystems increase with higher temperatures in quantitatively similar ways, consistent with similar enzymatic activation energies for organismal respiration (~0.65 eV). It has been observed that short-term (hours to days) and long-term (weeks to years) effects of warming might deviate from this model, and that acclimatisation of respiration and gross primary production might occur to global warming (Allen *et al.* 2005, Dewar *et al.* 1999, Lopez-Urrutia *et al.* 2006, Yvon-Durocher *et al.* 2012).

Increases in net primary production (NPP) and respiration (ER) at higher temperatures are also expected to occur within ecosystems, concurrent with change in phytoplankton community composition, body size and resource use. For example Lewandowska *et al.* (2012) documented increased NPP and ER with warming, accompanied by decreases in phyto- and zooplankton biomass (Lewandowska & Sommer 2010, Sommer & Lengfellner 2008), increased grazing pressure, changes in autotroph:heterotroph ratios (Lewandowska *et al.* 2012, O'Connor *et al.* 2009), and cell sizes decrease with warming (Daufresne *et al.* 2009, Sommer & Lengfellner 2008, Winder & Sommer 2012). Atkinson *et al.* (2003) postulated in their Temperature-Size–Rule (TSR) that ectothermic animals will decrease in body size with increasing temperatures by a value of 2.5% per °C. Such size shifts could reflect system-level metabolic constraints of temperature (Yvon-Durocher *et al.* 2012). Evidence for ecosystem and community shifts in response to warming have been documented by distinct empirical tests and formulated by independent theories, and we still lack a coherent exploration of joint effects of temperature on ecosystem and community responses. Such a synthetic study is needed to begin to understand whether one thermal response constrains the others.

These previous tests and the MTE do not consider whether a predator could alter the temperature dependence of ecosystem fluxes. However, trophic cascades can be very important for affecting species diversity, size structure and even carbon flux (Carpenter & Kitchell 1996, Schindler *et al.* 1997). By altering the energy flow through systems and the allocation of biomass, the presence of consumers could constrain ecosystem or community responses to temperature. The macroecological accuracy of MTE suggests that consumer presence does not affect the temperature dependence of ecosystem fluxes for comparisons among systems (Yvon-Durocher *et al.* 2012), and there is no theoretical prediction that it should. However, the temperature driven responses of a community have consequences for all linked trophic levels. Grazing impacts of herbivores on phytoplankton and predation on zooplankton affects phytoplankton biomass differently. Thus, only presence of herbivores has a stronger top-down effect on phytoplankton, whereas additional predators presence can release the grazing pressure of herbivores on phytoplankton.

Here, we approach this problem by focusing on how environmental warming affects two key ecosystem processes – net primary production and respiration - by testing MTE through effects on size/mass dependence at the community level under three distinct trophic scenarios. We manipulated temperature and measured responses of ecosystem fluxes concurrently with body size, predation, and grazing and their influence to metabolic balance shifts. We asked 4 fundamental questions:

(a) Does the temperature dependence of net primary production and ecosystem respiration depend on food chain length?

(b) Do mass-specific temperature effects on oxygen fluxes vary with increasing food chain length?

(c) How does temperature affect community structure, in terms of body size, abundance, and age distribution?

(d) How do ecosystem oxygen fluxes differ between long-term (inclusive bloom dynamics) and short term (settled bloom dynamics) analyses of ecosystems?

To answer these questions we combine a whole-system experiment with a 10 °C temperature gradient and 3 trophic structures (w/ predator, w/o predator, and w/o herbivore), allowing us to quantify the functional relationship between temperature and community structure and function in an aquatic food web.

Methods

We manipulated temperature in artificial freshwater ponds. We set up a gradient of temperatures that spanned 10 °C to test the following three hypotheses: a) temperature positively affects net primary productivity and community respiration rates, and the rate of increase is consistent with the activation energies of E = 0.32 and E = 0.65, respectively, b) ecosystem-level slope does not depend on food chain length, but c) the response of biomass and community structure to temperature does depend on initial trophic structure. Finally, we hypothesized that if the changes in community structure are constrained by the effects of temperature on fluxes, the changes in flux are not different over long time scales but stabilized systems ecosystems have different fluxes at short periods.

Mesocosm experiment

We tested the effects of temperature (10 levels) and food chain length (3 levels) in a factorial freshwater mesocosm experiment consisting of 30 experimental units. This regression design was chosen to model the response of the community to a temperature and to test for interactions with predation.

The outdoor mesocosms (370 L tanks) were situated on a gravel pad at the University of British Columbia, Vancouver, Canada. Mesocosms were filled with municipal water and left for one week after filling to allow chlorine to evaporate before the organisms were introduced (Kratina *et al.*, 2012). We added 160 µg $NaNO_3L^{-1}$ and 10 µg KH_2PO_4 L^{-1} to each tank, resulting a ratio of 16:1 (N:P). Temperatures were maintained using submersible aquarium heaters (50, 100, 150, 200, 250, 300, 350, 400, 450 Watt) to increase water temperature above ambient daily and seasonal temperature fluctuation of unheated tanks respectively. Thermochron ibutton data loggers were used to ensure that the heaters maintained a constant temperature difference between warmed and ambient tanks monitored the temperature over the course of the experiment (measurement intervals every 30 min). Water levels were maintained throughout the experiment by frequent natural precipitation and weekly addition of equal volumes of filtered water to each tank once a week.

The trophic treatments were: one trophic level consisted only of algae, two trophic levels of algae and herbivore consumers (copepods and cladocerans), and the three trophic level system of algae, herbivore consumers and backswimmers (Nototecidae) as predators. Mesocosms were inoculated with 1 L^{-1} of pondwater containing living phytoplankton, which was collected with and filtered through a 64 µm sieve to separate zooplankton and their larvae from phytoplankton. Three days after adding phytoplankton to mesocosms, zooplankton were collected at Trout Lake with a 64 µm mesh sized vertical colonial net, all net hauls were mixed to ensure similar species composition. Zooplankton was acclimated overnight to the mesocosm temperatures and dead organisms removed from the bucket bottoms. Two individuals of *Daphnia* sp. and copepods per liter were added per tank. The initial experimental communities consisted of a total pool of 8 phytoplankton and 3 zooplankton taxa. Covering each mesocosm with two layers of window screen minimized escape or colonization of other invertebrates.

We introduced 2 individual Notonectid predators on July 3rd, 2012 (experiment day 7) to initiate predation treatments. Notonectids have been shown to generate trophic cascades by supressing zooplankton in pond ecosystems (McArdle & Lawton 1979). To maintain the trophic structure treatment, we replaced Notonectids that died during the experiment with similar-sized individuals from the same source population.

During the experimental period of 10 weeks, we measured key biological, physical and chemical variables. Water temperature was measured daily. Mesocosms were sampled once a week for phytoplankton, chlorophyll, zooplankton, primary production and respiration.

Estimation of fluxes

Whole ecosystem NPP and ER rates were measured using the dissolved oxygen (DO) change technique with YSI-85 oxygen sensor (Yellow Springs Instruments, Yellow Springs, Ohio, USA) (Marzolf *et al.* 1994). The technique assumes that changes in DO concentration over a diel cycle represent the metabolic activity (photosynthesis and respiration) of the aquatic ecosystem.

The DO measurements were used to calculate NPP, GPP, and ER for each mesocosm. The change of DO (∆DO) was measured over 24 h. Oxygen concentration (mg/L) was measured at dawn, dusk and dawn on the following day. Photosynthetic dawn represented minimum O_2 concentration, after which we assumed all subsequent values were greater (Kratina *et al.*, 2012, Yvon-Durocher & Allen, 2012, Yvon-Durocher *et al.*, 2010). And photosynthetic dusk was defined as maximum O_2 concentration after which all subsequent values were assumed to be lower. Each value was assigned for day – or night-measurement.

NPP ($mgO_2\ L^{-1}\ h^{-1}$) = 24 * ((DOdusk – DOdawn1) / hours of measurement duration)

ER ($mgO_2\ L^{-1}\ h^{-1}$) = 24 * ((DOdawn2 – DOdusk) / hours of measurement duration)

Mass-specific NPP was calculated from total NPP divided by phytoplankton carbon biomass, estimated by chlorophyll a concentration multiplied by 55 mg carbon / mg chl a, and assuming no temperature dependence of the chlorophyll to carbon ratio (Gasol *et al.* 1997). Mass-specific ER was calculated from total ER values and then divided by total estimated community biomass (phytoplankton + zooplankton). To test whether temperature dependence of system-level and mass-specific fluxes were consistent with individual temperature dependences of metabolic rates (NPP and ER), we analysed the relationship

between the metabolic fluxes and 1/kT and compared slopes against the predicted value of E=0.65 (for ER) and E=0.32 (for NPP), respectively (Lopez-Urrutia *et al.* 2006, Allen *et al.* 2005).

Estimation of abundance and biomass:

We quantified phytoplankton biomass by estimating the concentration of chlorophyll a in 100 mL samples collected from the middle depth (~ 40 cm from surface) of each tank. Chlorophyll a data were used for the calculation of carbon content after Gasol *et al.* (1997). Phytoplankton community composition was assessed from 50 mL samples collected weekly and fixed with Lugol's iodine solution (5%) and counted by Utermöhl method (Utermöhl, 1958).

We also collected 10 L depth-integrated zooplankton samples once a week. Samples were filtered through a 64 µm sieve, the filtered water was returned to the mesocosms and the plankton samples were fixed with Lugol's iodine solution. Zooplankton organisms were identified to genus level and developmental stage for calanoid and cyclopoid copepods, and between mature and immature of *Daphnia* sp., counted under 10 x magnification.

Size

We measured the size (standard length) for each zooplankton individual counted (all developmental stages). Average individual size (standard length) and standard deviation for each community were estimated.

We estimated individual biomass with a length-mass regression after Vijverberg and Frank (1976):

$Ln(W) = ln(\alpha) + B*ln(L)$

For *Daphnia*: $ln(\alpha) = 1.468$ $B = 2.829$

For copepods: $ln(\alpha) = 1.821$ $B=0.654$ (Heinle & Flemer 1975); dry weight to carbon in µg was calculated by the multiplication by 0.5 after Berberovic (1990). We assumed that these parameters were temperature independent, or at least that any temperature dependence was minor relative to changes in density or length.

Statistical Analysis

To estimate the effects of warming on total NPP and ER, mass-specific NPP, and chlorophyll a concentration through time, we constructed linear mixed-effect models (LME) in which temperature, trophic level, and time were fixed independent variables. We treated individual mesocosm as a random factor. For each dependent variable, we ranked models using Akaike's Information Criterion (AIC), which takes into account both goodness of fit and model complexity.

Analyses for mass-specific NPP and ER of week 8, body sizes and abundances were performed with generalized linear models (GLM). All response variables were ln-transformed prior to analyses to achieve normality of residuals and metabolic rate activation energies by plotting on Arrhenius scale.

We computed all statistical tests in R statistical software (R Developmental Core Team 2006) with the packages nlme, MuMIn, and qpcR.

Results

Metabolic rates

Total ER and NPP

Total NPP and ER were affected significantly by temperature (Tab. 4.1, Fig. 4.1A), and increased with warming. Our experimental results of the total NPP over time showed that the interaction of time and temperature had a significant effect on the total NPP. Interestingly, our results showed that food chain length significantly affected the activation energy of total NPP: the activation energy of total NPP was lower when consumers and predators were present (Tab. 4.1).

Tab. 4.1 Results of best fitting linear-mixed models explaining the effects of temperature on ecosystem fluxes, zooplankton abundance and phytoplankton biomass (values in bold are significant at $p < 0.05$).

Variable	Factor	Value	Std. Error	dF	t-value	p-value
Ln (total NPP)	Intercept	90.329	9.891	205	9.132	**<0.01**
	PZ	-28.188	7.443	27	-3.787	**<0.01**
	PZN	-22.722	7.511	27	-3.025	**<0.01**
	invT	-0.391	0.254	205	-9.101	**<0.01**
	Week	-6.860	1.396	205	-4.915	**<0.01**
	PZ : invT	0.138	0.190	205	3.793	**<0.01**
	PZN : invT	0.057	0.191	205	3.048	**<0.01**
	invT : week	0.177	0.341	205	4.924	**<0.01**
Ln (total ER)	Intercept	28.276	6.405	207	4.415	**<0.01**
	PZ	-10.829	8.627	27	-1.255	0.22
	PZN	-26.904	8.664	27	-3.105	**<0.01**
	invT	-0.712	0.164	207	-4.353	**<0.01**
	PZ : invT	0.287	0.221	207	1.300	0.19
	PZN : invT	0.687	0.221	207	3.104	**<0.01**
Ln (Phytoplankton biomass +1)	Intercept	-5.022	4.995	204	-1.005	0.32
	PZ	-5.349	3.522	27	-1.519	0.14
	PZN	-1.616	3.523	27	-0.459	0.65
	invT	0.171	0.129	204	1.326	**0.02**
	PZ : invT	0.492	0.478	204	1.028	0.31
	PZ : invT	0.134	0.090	204	1.486	**0.05**
	PZN : invT	0.046	0.090	204	0.505	0.06
	invT : week	0.015	0.012	204	-1.237	0.22
Ln (temporal mass-spec. NPP)	Intercept	59.794	24.023	201	2.489	**0.01**
	PZ	-109.462	32.416	27	-3.377	**<0.01**
	PZN	-37.717	34.404	27	-1.906	0.28
	invT	-1.695	0.617	201	-2.746	**<0.01**
	Week	-2.357	3.535	201	-0.667	0.51
	PZ : invT	2.867	0.835	201	3.435	**<0.01**
	PZN : invT	0.984	0.888	201	1.108	0.27
	PZ : week	3.307	4.481	201	0.738	0.46

	PZN : week	-0.471	4.460	201	-0.106	0.92
	invT : week	0.068	0.091	201	0.747	0.46
	PZ : invT : week	-0.095	0.115	201	-0.825	0.41
	PZN : invT : week	0.008	0.114	201	0.68	0.95
Ln (mass-spec. NPP week 8)	Intercept	20.201	9.794	28	2.062	**0.05**
	invT	-0.496	0.247	28	-2.005	**0.05**
	PZ	16.005	15.568	28	1.028	0.31
	PZN	2.432	15.433	28	0.158	0.87
	invT : PZ	-0.102	0.393	28	-1.034	**0.03**
	invT : PZN	-0.068	0.389	28	-0.176	**0.05**
Ln (mass-spec. ER week 8)	Intercept	79.722	27.831	25	2.865	**<0.01**
	PZ	-16.808	45.565	25	-0.369	0.72
	PZN	38.272	43.175	25	0.886	0.38
	invT	-2.135	0.703	25	-3.039	**<0.01**
	invT : PZ	0.466	1.151	25	0.405	0.69
	invT : PZN	-0.958	1.091	25	-0.878	0.39
Ln (Z abundance +1)	Intercept	-13.678	24.975	81	-0.548	0.59
	PZN	-3.407	11.084	40	-0.307	0.76
	invT	0.349	0.642	81	0.544	**0.05**
	Week	1.872	3.412	81	0.549	0.59
	invT : PZN	0.082	0.283	81	0.289	0.77
	invT : week	-0.044	0.087	81	-0.499	0.62

Total NPP activation energy of treatments with only phytoplankton was 0.39 eV (±2.31 CI). Food chains with herbivores and predators were not significantly different from each other, but two trophic level food chains were significantly different to phytoplankton only treatments (Tab. 4.1). Two trophic level systems had an activation energy of 0.25 eV (±0.72 CI), and three trophic levels 0.33 eV (±0.58 CI). The AIC model selection indicated that trophic chain length has to be included into the statistical analysis (Tab. 4.2). Results of the linear mixed model with temperature as the only explanatory variable indicated that temperature significantly increased total NPP over the total experimental period and has activation energy of 0.39 eV (Tab. 4.2, Tab. 4.3).

Tab. 4.2 Akaike model selection results

Variable	model	δ_{AIC}	Rel.LL	weights
Ln (NPP)	lme(log(NPP)~trophic.level*invT*week, random=~1|Tank)	0.000	1.000	0.7357
	lme(log(NPP)~trophic.level*invT, random=~1|Tank)	10.544	0.005	0.0038
	lme(log(NPP)~invT, random=~1|Tank)	15.602	<0.000	0.0003
	lme(log(NPP)~trophic.level*invT+ week*invT, random=~1|Tank)	2.069	0.355	0.2611

Ln (ER)	lme(log(ER)~trophic.level*invT*week, random=~1|Tank)	27.484	1.076e-6	9.805e-1
	lme(log(ER)~trophic.level*invT, random=~1|Tank)	0.000	1.000+e0	9.111e-1
	lme(log(ER)~invT, random=~1|Tank)	6.133	4.658e-2	4.244e-2
	lme(log(ER)~trophic.level*invT+ week*invT, random=~1|Tank)	5.951	5.103e-2	4.649e-2
Ln (mass-specific NPP)	lme(mass NPP~trophic.level*invT*week, random=~1|Tank)	0.000	1.000	0.998
	lme(mass NPP~trophic.level*invT, random=~1|Tank)	15.639	4.107e-4	4.107e-4
	lme(mass NPP~invT, random=~1|Tank)	14.032	8.997e-4	8.961e-4
	lme(mass NPP~trophic.level*invT+ week*invT, random=~1|Tank)	17.949	1.264e-4	1.264e-4
Ln (phytoplankton biomass +1)	lme(P biomass~trophic.level*invT*week, random=~1|Tank)	26.517	1.746e-6	1.746e-6
	lme(P biomass ~trophic.level*invT, random=~1|Tank)	46.104	9.741e-11	9.741e-11
	lme(P biomass ~invT, random=~1|Tank)	33.497	5.325e-8	5.324e-8
	lme(P biomass ~trophic.level*invT+ week*invT, random=~1|Tank)	0.000	1.000+e0	9.999e-1
Ln (zooplankton abundance +1)	lme(Z abundance +1~trophic.level*invT*week, random=~1|Tank)	10.352	0.006	0.005
	lme(Z abundance +1 ~trophic.level*invT, random=~1|Tank)	5.285	0.067	0.057
	lme(Z abundance +1 ~invT, random=~1|Tank)	0.000	1.000-e0	0.850
	lme(Z abundance +1 ~trophic.level*invT+ week*invT, random=~1|Tank)	4.546	0.103	0.088

Tab. 4.3 Linear-mixed model results only of temperature as dependent variable (values in bold are significant at $p < 0.05$).

Variable	Factor	Value	Std. Error	dF	t-value	p-value
Ln (total NPP)	Intercept	28.402	3.329	209	8.531	**<0.05**
	invT	-0.39	0.085	209	-8.447	**<0.05**
Ln (total ER)	Intercept	11.893	4.059	209	2.930	**<0.05**
	invT	-0.62	0.104	209	-2.794	**<0.05**
Ln (phytoplankton biomass)	Intercept	30.693	4.964	209	6.183	**<0.05**
	invT	-0.653	0.127	209	-5.133	**<0.05**
Ln (mass-specific NPP)	Intercept	-18.329	5.990	209	-3.059	**<0.01**
	invT	0.345	0.153	209	2.251	**<0.05**
Ln (mass-spec NPP week 8)	Intercept	25.460	6.405	28	3.975	**<0.01**
	invT	-0.631	0.162	28	-3.903	**<0.01**
Ln (mass-spec. ER week 8)	Intercept	1.714	0.574	28	2.987	**<0.01**
	invT	-0.042	0.015	28	-2.931	**<0.01**
Ln (temporal Z abundance +1)	Intercept	-11.696	5.847	81	-2.000	**0.05**
	invT	0.324	0.149	81	2.168	**<0.05**

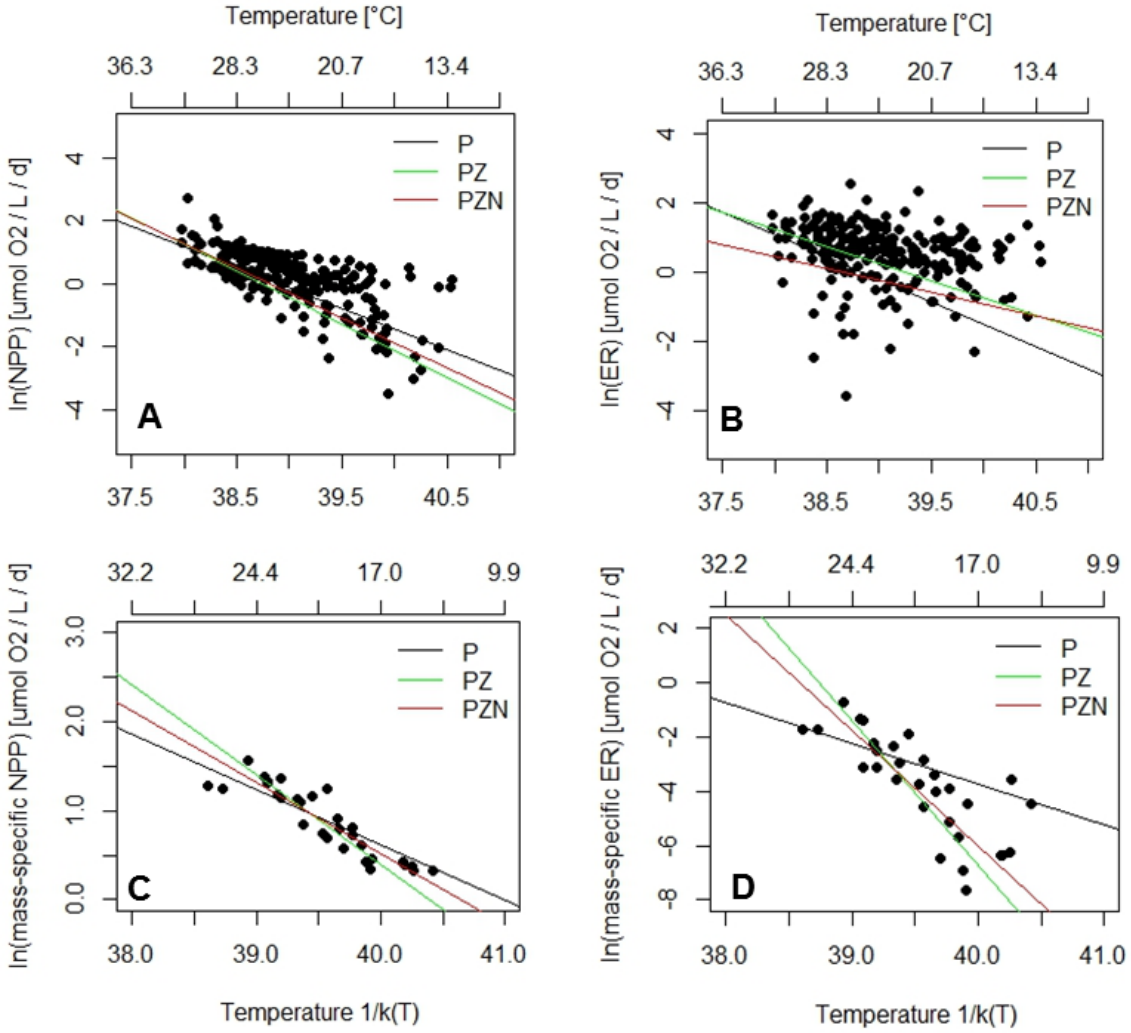


Fig. 4.1 Ecosystem metabolic rates: **A)** temporal total NPP, **B)** temporal total ER, **C)** mass-specific NPP week 8, **D)** mass-specific ER week 8

Total ER increased significantly with warming by activation energy of 0.72 eV (±1.18 CI, Tab. 4.1, Fig. 4.1B). Food chain length changed the activation energy of total ER significantly; the longer the food chains the lower the activation energy for total ER (two trophic levels 0.42 eV (±0.29 CI); three trophic levels 0.03 eV (±0.61 CI); Tab. 1). Treatments with phytoplankton as only trophic level had significantly higher total ER than treatments with herbivores and predators (Tab. 4.1). Total ER of systems with phytoplankton and zooplankton were significantly different to phytoplankton only systems (Tab. 4.1). The AIC selection indicated that time did not need to be included into the statistical analysis (Tab. 4.2). Results of the linear mixed model with temperature as the only explanatory variable indicated that temperature significantly increased total ER over the total experimental period (0.62 eV, Tab. 4.2, Tab. 4.3).

Mass-specific metabolic rates

Mass-specific NPP was significantly affected by temperature and had activation energy of 1.67 eV (±1.23 CI; Tab. 4.1). Food chain length decreased the activation energy of mass-specific NPP significantly at higher temperatures. The activation energy of phytoplankton-herbivore food chains was 1.17 eV (±1.0 CI). Activation energy of mass-specific NPP of a three trophic level system was not significantly different from phytoplankton only treatments (0.71 eV (±0.40 CI), Tab. 4.1). Results of the linear mixed model with temperature as the only explanatory variable indicated that temperature significantly increased mass-specific NPP over the total experimental period (0.35 eV, Tab. 4.2, Tab. 4.3).

Mass-specific NPP and ER of week 8 were significantly affected by higher temperatures, whereas mass-specific NPP was also affected by food chain length (Tab. 4.1, Fig. 4.1C). Higher temperatures increased mass-specific NPP and ER during week 8. Food chain length did only significantly affect mass-specific NPP but not mass-specific ER, but tended to decrease activation energies with food chain length (Tab. 4.1). Food chains with two trophic levels, phytoplankton and herbivores, had a higher activation energy of mass-specific NPP (0.59 eV (±2.08 CI)) than only phytoplankton treatments with 0.49 eV (±0.18 CI, Tab. 4.1). Interestingly, presence of predators increased the activation energy but not as high as in the two trophic level system, 0.51 eV (±1.02 CI, Tab. 4.2). Results of the linear mixed model with only temperature as explanatory variable led to the suggestion that higher temperature significantly enhanced mass-specific NPP (Tab. 4.3). The activation energy of mass-specific NPP was 0.63 eV independently of trophic chain length (Tab. 4.3).

Mass-specific ER of week 8 significantly increased at higher temperatures (Tab. 4.1, Fig. 4.1D), also the interaction of food chain length and temperature affected mass-specific ER of week 8, but not significantly. The activation energy of only phytoplankton was 2.1 eV (±0.94, Tab. 4.1). Food chain length with two trophic levels and temperature decreased the activation energy of mass-specific ER (1.67 eV ± 2.4 CI). Three trophic levels with predators increased mass-specific ER to 3.1 eV (±0.91 CI). Results of the linear mixed model with only temperature as explanatory variable indicated that temperature significantly increased mass-specific ER over of week 8 (0.04 eV, Tab. 4.2, Tab. 4.3).

Phytoplankton

Phytoplankton biomass significantly decreased at higher temperatures (Tab. 4.4, Fig. 4.2A). The AIC suggested simpler mixed models with temperature, as only explanatory variable are the best fitting models (Tab. 4.2, Tab. 4.3), Time and trophic level did not affect phytoplankton biomass significantly (Tab. 4.1).

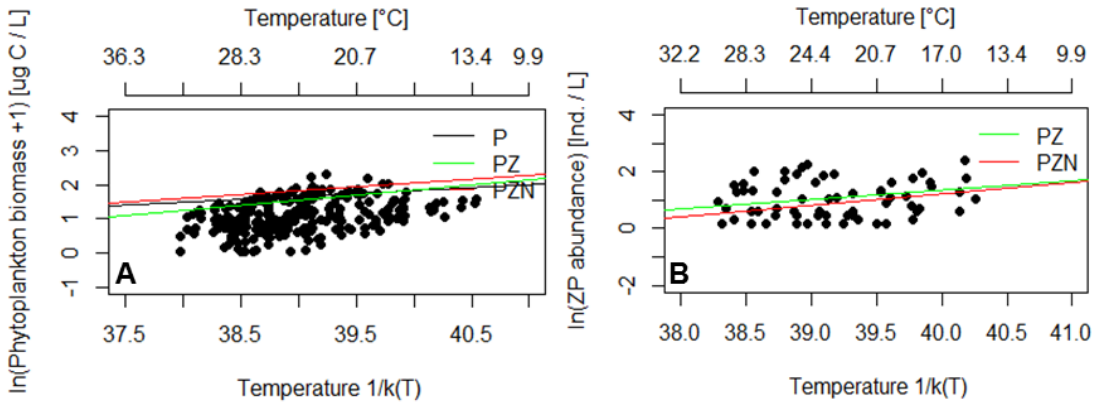


Fig. 4.2 A) Phytoplankton estimated carbon biomass of the experimental periode **B)** Zooplankton abundance over the experimental period (week 3- week 8) of cladocerans and copepods.

Tab. 4.4 Results of generalized linear models of temperature and food chain length on changing community structure parameters (values in bold are significant at $p < 0.05$).

Variable	Units	Factor	Estimate	SE	p-value
Zooplankton body size	µm	Temperature	0.322	0.000	0.75
		PZN	-3.475	0.072	**<0.01**
		Temp x PZN	0.150	0.000	**0.04**
Mature *Daphnia* sp. body size	µm	Temperature	-2.014	0.000	**0.05**
		PZN	2.902	0.136	**<0.01**
		Temp x PZN	-2.596	0.000	**0.01**
Heterotroph/Autotroph ratio		Temperature	3.058	0.086	**0.03**
		PZN	-0.207	31.228	**0.04**
		Temp x PZN	1.868	0.107	**0.05**
Total zooplankton abundance	Ind L^{-1}	Temperature	1.406	0.143	**<0.01**
		PZN	-15.351	4.581	**<0.01**
		Temp X PZN	0.619	0.202	**<0.01**
Adult zooplankton abundance	Ind L^{-1}	Temperature	-0.028	0.002	**<0.01**
		PZN	-4.030	0.594	**<0.01**
		Temp x PZN	0.015	0.003	**<0.01**

Zooplankton

All occurring zooplankton species were identified as cladocerans of the genus *Daphnia* sp. or copepods (calanoid and cyclopoid taxa *Eurytemora* sp. and *Cyclops* sp.). Tanks with missing zooplankton of week 8 were excluded from the data- and statistical analysis. We suggest that

consumers might have died during the time course of the experiment because zooplankton occurred the weeks before in tanks with missing zooplankton data.

The ratio of copepod-to-cladoceran abundance showed that more copepod taxa were abundant under predation pressure (see Appendix Table 4.1).

Abundance

Zooplankton abundance over time was only significant affected by temperature (Tab. 4.1, Tab. 4.2, Tab. 4.3). Tanks with higher temperature had lower abundances than tanks with lower temperature (Fig. 4.2B).

In week 8, total zooplankton abundance significantly decreased at higher temperatures (Tab. 4.4, Fig. 4.3B). The presence of predators significantly decreased total abundance and the interaction of temperature and predators was also significant (Tab. 4.4). Copepod and cladoceran abundance in the three trophic level systems was significantly lower compared to treatments with less trophic levels (Tab. 4.4, Fig. 4.3B). The interactive effect of temperature and food chain length was significant (Tab. 4.4). Within our total zooplankton abundance data, each trophic level treatment had one missing data point. Total zooplankton abundance could not be counted for two trophic level systems at 27 °C and for three trophic level systems at 22 °C due to missing zooplankton individuals. Copepods and cladoceran abundance estimates that excluded larvae had missing values in the treatments of two trophic levels at 19 °C and 27 °C, as well as in three trophic level treatments at 22 °C.

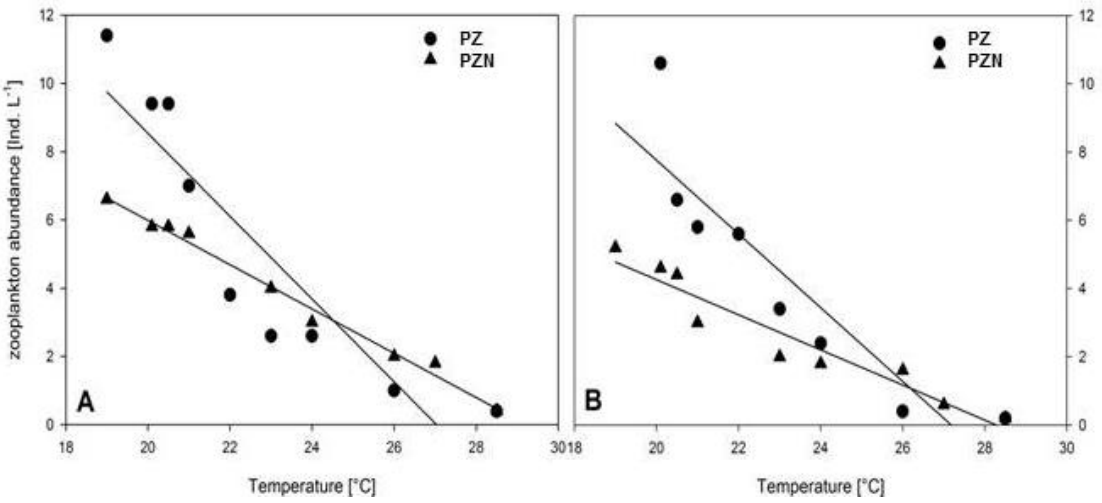


Fig. 4.3 A) total zooplankton abundance (including all stages and larvae) **B)** zooplankton abundance of adult zooplankton (copepods and Daphnia).

Body Size

Average body size of the entire zooplankton community was affected by temperature and the interaction of temperature and food chain length (Tab. 4.4). The presence of predators significantly decreased community body size. Community body size represents the body size

of all occurring zooplankton taxa, irrespective of developmental stage. Community size structure shifted from the larger taxon *Daphnia* sp. to smaller copepod species under predation and temperature (Appendix Fig. 4.1)

Temperature negatively affected mature *Daphnia* sp. mean body size (Tab. 4.4, Fig. 4.4A, B). The body size of adult *Daphnia* sp. decreased more strongly when Notonectids were present than when *Daphnia* was the highest trophic level at higher temperatures (Tab. 4.4, Fig. 4.4B). Size data for mature *Daphnia* sp. was missing for two trophic level systems at 19°C and 27 °C, as well as for three trophic level systems at 20 °C and 27 °C. Data of community body size were missing for 2 trophic level systems at 19 °C and 27 °C, and for three trophic level systems at 22 °C.

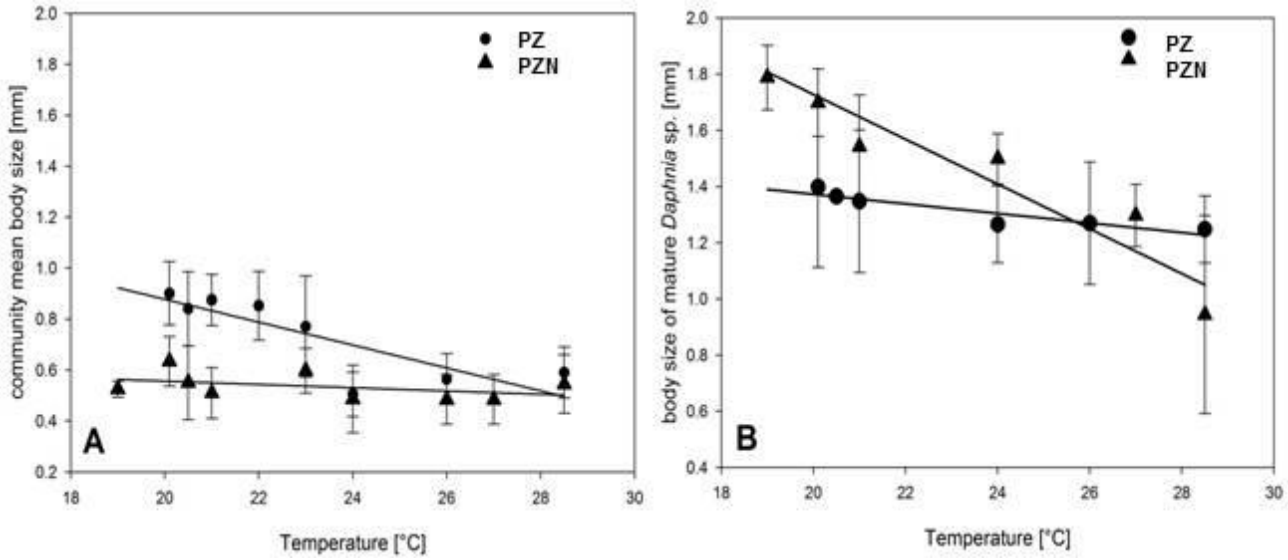


Fig. 4.4 A) Zooplankton community size (includes all body sizes of copepods and cladocerans) (mean values with standard deviations) B) Mean body size of mature *Daphnia* sp. of each treatment (with standard deviations) of week 8 samplings.

Discussion

Results of our mesocosm study suggested that higher environmental temperatures decreased the production of organic carbon via lower phytoplankton biomass and related mass-specific primary production whereas carbon loss by respiration increased. Grazing by herbivores decreased phytoplankton biomass and total NPP whereas community respiration increased at higher temperatures. Predation by Notonectids on herbivores dampened the effect of grazing by zooplankton but still led to lower phytoplankton biomass and primary production and higher community respiration.

Our experimental setup was able to implement realistic climate change predictions of higher water temperatures within a mesocosm study. This approach allowed us to test for relative

strength and interaction of physically and biologically mediated rising water surface temperature on a plankton community and the impacts with predation pressure. Kratina *et al.* (2012) found that communities are more susceptible to the cascading effects of predators with higher environmental temperature. They suggest that higher temperatures shift primary production towards stronger top-down control, which we were able to show during our experiment. But additionally, we were able to detect differences in temperature-driven flux changes between producers, consumers, and predators. Predation of Notonectids on zooplankton led to intermediate flux changes.

Yvon-Durocher *et al.* (2010) found that temperature dependences of ecosystem respiration and primary production of a whole system are fundamentally, and quantitatively consistent with different activation energies at the individual level. In our experiment, total respiration and net primary production rates are closely related to Yvon-Durocher *et al.* (2010) predictions, but just in systems with phytoplankton as the only trophic level. Yvon-Durocher *et al.* (2010) suggested that different temperature sensitivities of respiration and production are occurring because larger fractions of fixed carbon by photosynthesis are remineralized and released as CO2. Interestingly, grazing by herbivores, and predation by Notonecids on herbivores, leads to a decrease of system-level activation energies of total net primary production with temperature. We suggest that the differences with increasing food chain length occur because the total biomass of the system is also changing, reflecting shifts in abundance and body size of producing and respiring organisms in the systems (Yvon-Durocher & Allen 2012)(see Fig. 2, Fig. 4). Results of our study show that phytoplankton biomass, abundance, and sizes of herbivores are decreasing with increasing temperatures. These results are in line with other mesocosm studies with freshwater and marine organisms where algae biomass decline was observed with increasing temperatures (Daufresne *et al.* 2009, Kratina *et al.* 2012, Lewandowska & Sommer 2010). Several studies, experimental and meta-analyses showed that higher temperatures affect phytoplankton by shifts to smaller cell size and changes to smaller species (Daufresne *et al.* 2009, Peter & Sommer 2012, Sommer *et al.* 2012). Additionally, Garzke et al (2014) have observed temperature effects on zooplankton, where marine copepods had smaller size-at-defined developmental stage and lower abundances at higher temperatures.

Faster enzymatic activity and faster metabolism increase the energy demands of the individual. The individual organism has to cover the increasing energy demands for staying alive and healthy (Claireaux & Lefrancois 2007). Higher food uptake of zooplankton and predators lead to a change of the community. The herbivore-to-autotroph ratio significantly increased with warming, because of stronger top-down control of consumers on phytoplankton with warming, which was firstly suggested by Müren *et al.* (2005) and additional predation of Notonectids on *Daphnia* sp. led to significantly smaller body sizes to mature earlier at smaller size to escape predation pressure. The observed changes in body size are occurring within the context of net primary production and respiration changes. Brown et al. (2004) stated in the ‘Metabolic Theory of Ecology’ that enzymatic driven ecological processes are directly affected by changing temperature and that changes on the individual level have consequences in the ecology of a community and population.

Temperature driven decline of phytoplankton and zooplankton biomass have to be incorporated into carbon flux studies. Yvon-Durocher and Allen (2012) tested mass-specific changes of carbon flux with a mesocosm experiment. They observed that small size changes are able to change the biochemical cycling rates. Their study supports our observations that mass-specific fluxes match closely earlier predictions of activation energies of ecosystem

production and ecosystem respiration. Primary production and respiration rates are significantly correlated with community size in ambient and high temperatures (Yvon-Durocher & Allen 2012). Further on, predation on herbivores reduces the grazing or indirect temperature effects on phytoplankton. Algae biomass decline is lower at warmer temperatures in food chains with predators compared to only herbivorous grazing dependent food chains.

Predation on herbivore consumers leads to intermediate responses of the food web to warming. Predation reduces the number of phytoplankton consumers and dampens the grazing pressure on phytoplankton. Declining abundance of mature *Daphnia* sp. is related to predation on larger bodied individuals with increasing temperature (Kratina *et al.* 2012). But still, warming strengthens the top-down control independent of food chain length, but is able to dampen the magnitude of decreasing biomass, and algae blooms are able to re-occur.

Our long term results of phytoplankton biomass showed that in ambient temperatures grazing of herbivores and predation on grazers have a significant effect on algal biomass. It could be observed that blooms appeared in two and three trophic level systems during the experimental period of 8 weeks. The fluctuations could appear due to higher amounts of nutrients by recycled organic matter. Warming showed a significant lower phytoplankton biomass but treatments only with algae showed smaller blooms whereas grazing and predation treatments had significantly algae biomass and stabilized over time. These results are in line to theoretical predictions of O'Connor *et al.* 2011). Grazing and warming might lead to lower biomass and consequently lower resource supply for higher trophic levels and also lower particulate organic matter for nutrient recycling.

We conclude that responses of the individual level, smaller body size and lower abundances have to be included into ecosystem analysis and the impact of higher temperatures on ecosystem metabolic balances. The ecosystem carbon flux might be more affected at higher temperatures than earlier stated, because temperature responses of the individual organism and the interactions between trophic levels have to be included into the analysis. Stabilized ecosystems, here our results of week 8, showed that higher temperatures affect more mass-specific NPP, irrespectively of food chain length than earlier studies suggested (Brown *et al.* 2004). Ecosystem respiration was 3 times higher at higher temperatures and more sensitive to warming than NPP.

GENERAL DISCUSSION

Predicting biological responses of the individual organism and ecosystems to global change remains a major challenge in ecology. This thesis contains results of novel experimental approaches testing new hypotheses concerning the impact of warming on the individual copepod size and abundances within a natural community structure (chapter 1), the combination of temperature and phosphorus availability on body size, developmental- and somatic growth rates (chapter 2), and the combined impacts of warming and ocean acidification on copepod prosome length, abundance and fatty acid composition (chapter 3). Furthermore, of the gained knowledge about temperature-related decrease of marine copepod body size, abundance, and biomass were extended to the ecosystem fluxes and food web interactions of natural limnic communities including predators of zooplankton (chapter 4). Overall, the results of this work suggest that different environmental factors of predicted global change firstly affect the individual level by changing metabolism, size, survival, and reproduction. These changes subsequently affect the ecosystem by decreasing biomass built-up and food quality, which have the potential to prevail impacts on higher trophic levels. In order to test the consequences of changing environmental conditions, it is mandatory to understand and identify the extent of changes in the individual within a community and their interactions as major drivers of the affected ecosystem.

Temperature as an ecological factor for zooplankton

Overall, the results of the experiments presented in this thesis have provided a picture of the role of temperature as a steering factor for growth and abundance of zooplankton. Indeed, temperature showed the strongest effects of copepod size and abundance in my experiments. Although nutrient stoichiometry (chapter 2) and acidification (chapter 3) have been shown to impact aquatic organisms, but their direct effects on copepods were less notable than temperature effects. In the experiments I present here, their most likely operation mode being indirect effects on copepod growth and metabolism through altered food quality and quantity of phytoplankton. Yet, my results show the extents of these indirect impacts are weaker than temperature effects in the focal plankton communities. Here, controlled semi-natural food web experiments were able to detect the importance of temperature changes on copepod body size and abundance. A mono-culture experiment was conducted to detect the possible reasons of decreasing body sizes of copepods under warming.

I showed in this study that changes of temperature alone had a strong impact on copepod body size and abundance (chapter 1). In agreement with the Temperature-Size-Rule (Atkinson 1994), I was able to show that body sizes of adult copepods and size-at- a defined stage of *Acartia* sp. were smaller at higher temperatures. Additionally, I could show that copepod abundances were lower at higher temperatures. In my mesocosm studies, I did not consider nutrient limitations, because nutrient concentrations in each experiment were high enough to guarantee non-limited phytoplankton growth during the start phases of algal blooms. The natural species composition and density of possible food sources as starting conditions for the experiments allowed me to observe the different responses and food web

interactions between copepods and lower trophic levels under naturally encountered nutrient conditions.

Similar studies for marine ecosystems could help to understand how temperature-related smaller body sizes and lower abundances may affect food webs in response to predicted climate warming. The evaluation of the interplay of different ecological stressors, e. g. carbon-to-phosphorus ratio (chapter 2) or acidification (chapter 3), and temperature is the first step to compare different factors affecting copepods.

Direct temperature effects on copepod metabolism

The predicted increase of sea surface temperatures has a direct effect on organismal metabolism. Basal physiological and biochemical laws (Boltzmann 1872; Arrhenius 1889) show the temperature-dependence of enzyme activity, which lead to a faster metabolism. The mono-culture experiment (chapter 2) confirmed that temperature directly increases respiration, as stated by the metabolic theory of ecology (Brown *et al.* 2004), which predicts an increase of metabolic processes with increasing temperature.

My studies indicate that direct temperature impacts lead to faster development (chapter 1-3), higher respiration rates (chapter 2), and stage-specific mortality (chapter 1). It is known that temperature more strongly affects heterotrophic processes and that consumer activity increases with warming (O'Connor *et al.* 2009). Higher activity increases the energy demands of the individual to uphold metabolic processes under warming, which results in higher feeding rates to increase energy uptake. Even if copepods are able to partially compensate the direct temperature effects on growth when food is more abundant (chapter 3), body size still remains smaller at higher temperatures. Additionally, I could show that higher phosphorus availability is an important factor to support faster transition from stage-to-stage, but that copepods still have smaller body sizes at higher temperatures (chapter 2). Phosphorus limitation only prolongs the time to reach maturity at a (temperature-driven) smaller size.

In chapter 4, I illustrated that earlier observed decline of phyto- and zooplankton biomass with warming changes the mass-specific metabolic fluxes in a food-web more strongly than was suggested earlier for entire ecosystems (Allen *et al.* 2005). Plasticity in the interactions between trophic levels with each other (producers, consumers, and predators) is able to dampen the temperature-induced increase of mass-specific ecosystem net primary production and respiration. Mass-specific enzyme activation energies for ecosystem net primary production and respiration differ from observed individual-level photosynthesis and respiration. I could show that community size structure and abundance significantly influence fluxes and these results aid in understanding links between individual organisms and biogeochemical cycles. Temperature might act indirectly on zooplankton due to processes like release of faeces and DOC of copepods but also changes of aggregation and sinking (Piontek *et al.* 2009), might affect biogeochemical cycles, but which were not discussed in my thesis.

I could not observe shifts in zooplankton species composition or species richness with warming. The experimental mesocosm plankton communities were quickly dominated by few key-players, which perform well under laboratory conditions, such as *Acartia* sp. (chapter 1) or *Paracalanus* sp. (chapter 3).

Compositional shifts of copepods, which are the main consumers of phytoplankton >10 µm, might also affect phytoplankton species composition by changes in feeding preferences (Stibor *et al.* 2004).

The ongoing increase in ocean temperature and acidification will likely lead to shifts in zooplankton size, nutritional quality and abundance. This leaves the question open how ecosystem functioning (biomass transfer to higher trophic levels, overall biomass build-up and respiration) will be affected, because ecosystem functioning depends greatly on the functional characteristics of organisms presented in ecosystems and the distribution, abundance and their interactions over space and time.

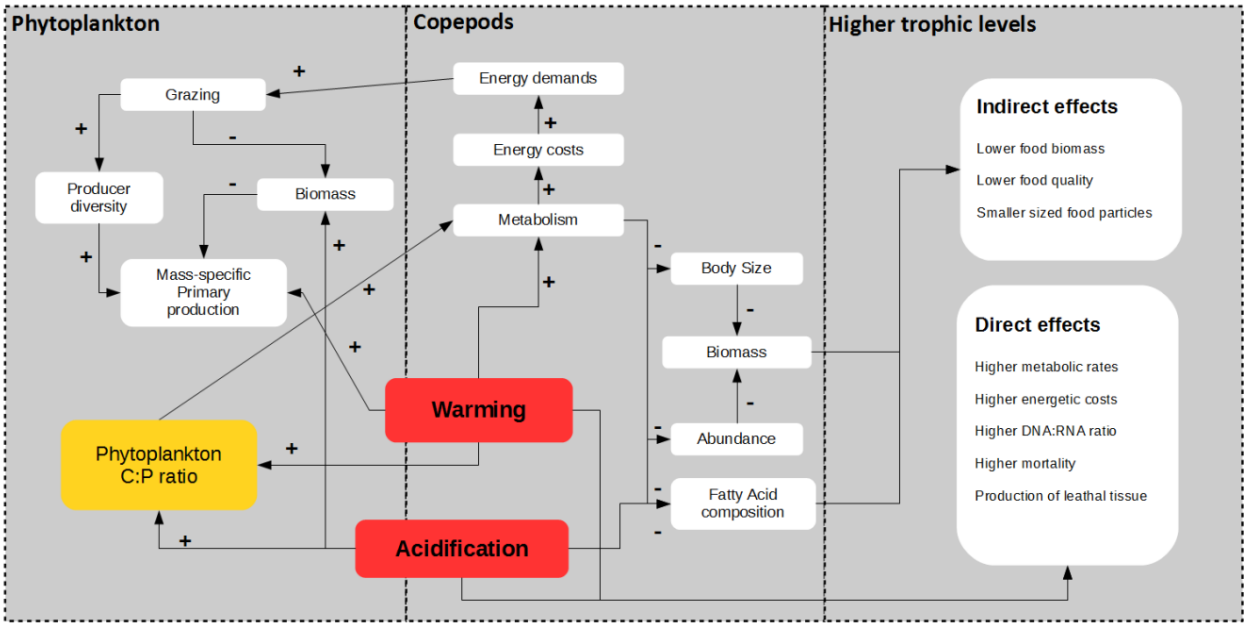


Fig. 3 Schematic temperature, P-stoichiometry and acidification impacts on biotic interactions in aquatic plankton communities. Minus represents a negative and plus a positive relationship. PP is the biomass specific primary productivity.

A summary of the zooplankton parameters, which I tested experimentally using mesocosm facilities in Kiel, Germany, and Vancouver, Canada, is presented in figure 3. I included temperature as the sole abiotic factor in this schematic food-web for better clarity. Additionally, I set the responses of the copepod community against the possible resulting effects on lower (phytoplankton) and on higher trophic levels, e.g. fish larvae. Possible consequences on phytoplankton and fish are in the following supported with results from the literature.

From this schematic, a conceptual model can be proposed, which is complete only for predictions of temperature effects. Temperature directly affects the metabolism of copepods, as described in chapter 2 and shown by Brown *et al.* (2004). Resulting from higher metabolic rates, energy costs and demands increase, which have to be covered by increased grazing activity on lower trophic levels (chapter 1, 3, 4), as stated in the metabolic theory of ecology (Brown *et al.* 2004) and empirically shown by O'Connor *et al.* (2009).

In accordance with the results shown in chapter 4, consumers have a negative effect on phytoplankton biomass which was also reported by Sommer and Lewandowska (2010). Lewandowska *et al.* (2014) reported that grazing of herbivore consumers has a positive impact on mass-specific primary production, because consumers increase producer diversity. But I could also show in chapter 4 that mass-specific net primary production increases with higher temperature and the presence of grazer and also additional presence of predators. This result indicates that higher temperatures increase the metabolic rates in such that the temperature driven decline in biomass does not reflect in mass-specific primary production.

Warming negatively affects copepods by increasing metabolic rates and causing higher enzyme activity, as stated in the metabolic theory of ecology (Brown *et al.* 2004). Lower survival rates, lower egg production and lower hatching rates lead to the lower copepod abundances at higher temperatures observed in chapters 1 and 2. The different temperature sensitivities of development (faster at higher temperature) and somatic growth (less enhanced at higher temperatures) of copepods consequently result in smaller adult body sizes. I could show this discrepancy between the two growth parameters in chapter 2. Smaller body sizes and lower abundances lead to lower biomass, which might affect higher trophic levels by decreasing food quantities. The fatty acid composition of copepods showed a lower quality by lower amounts of essential fatty acids at higher temperatures. Adverse changes in fatty acid composition can negatively affect fish development and growth. Malzahn *et al.* (2007) tested in a tri-trophic food chain how food quality can affect fish larvae condition and found that mineral nutrient requirements of fish larvae first have to be satisfied before growth can be promoted. In chapter 4, I could show that predation on zooplankton dampens the grazing effect on phytoplankton, and consequently the temperature effect on mass-specific metabolic fluxes.

Direct effects of warming and acidification on fish performance are well investigated. Warming and acidification individually affect fish larvae by lower RNA concentration at hatching, decreased protein biosynthesis (Franke and Clemmensen 2011), less stored lipids (Todd *et al.* 2007) and lethal tissue damages (Frommel *et al.* 2012). But indirect effects of warming, acidification and P-limitation due to less available food biomass (copepods) with changed nutritional composition (nutrient stoichiometry and fatty acid composition) need further studies.

Multi-stressor effects on copepod responses

The IPCC reports of the years 2007 and 2014 predict an increase of the global average sea surface temperatures and due to the uptake of CO_2 a decrease of ocean pH. Thus, warmer and acidified oceans may result in additional changes that act as stressors on organisms. Regional changes can be changes in surface salinity, stratification, nutrient availability, and oxygen depth profiles. Abiotic and biotic stressors usually do not operate independently, but rather often interact to produce combined impacts on biodiversity and ecosystem functioning (Frost *et al.* 1999; Schindler 2001).

Only scarce knowledge exists on combined effects of multi-stressors responses on individuals and communities. The development of a general theory of multiple stressors has hardly begun (Folt *et al.* 1999; Vinebrooke *et al.* 2004; Townsend *et al.* 2008). It is most important to understand the circumstances where joint impacts of multiple-stressors produces complex as opposed to simple responses.

Folt *et al.* (1999) class simple responses of organisms as additive and multiplicative, whereas complex outcomes are synergistic or antagonistic. In my thesis, I tested combined effects of temperature and phosphorus limitation on copepod growth (chapter 2) and the combined effects of temperature and acidification on copepod body size, abundance, and fatty acid composition (chapter 3).

The results of chapter 3 showed that phosphorus limitation itself leads to lower zooplankton developmental rates, and combined with higher temperatures, the difference between faster developmental rates and increased demands of phosphorus for growth lead to dissimilarities between lower somatic and developmental growth rates. Interestingly, the multiple-stressor response of copepods on P-limitation and warming does not lead to synergistic responses in smaller body sizes or mortality, but both stressors together lead to lower developmental times to reach smaller body sizes. Also, acidification and higher temperatures responses of chapter 3 were not additive or multiplicative as suggested by Pörtner (2008), but rather partially compensatory. In more detail, acidification had a partially compensatory effect on temperature-driven body size decrease and abundance decline. Multiple-stressor responses are difficult to interpret because changes can act directly on the individual (on the physiology) but also have indirect effects on the individual by e.g. available food biomass for compensation or changes in food quality. Especially, multi-stressor responses for natural communities are hard to distinguish because interactions between the different players of the food web can lead to compensatory effects. Of more general importance is my finding that the consequences of stressors are often unpredictable on the basis of knowledge of single effects. Given the increasing multitude of environmental stressors recognized to be associated with global change, there is an urgent need to develop a better understanding of the interactive effects of multiple stressors on ecosystems to better predict their responses to a changing environment.

Future perspectives

Based on the results of this thesis, several important questions are shaping out to be answered. Thus I suggest three future research directions, which may help to better understand zooplankton responses to climate change:

1. **Studies on body condition, fitness, reproduction success, and mortality of copepods under natural conditions.** In the studies discussed here, temperature effects on zooplankton were tested individually and in combination with P-limitation or acidification. This allowed me to take a first step in the assessment of multi-stressor impacts on copepod body size, nutritional quality and abundance. However, much more can be done in this field of research. The direct temperature and indirect food effects of multiple-stressors on copepod body condition and fitness (e.g. RNA:DNA ratio), reproduction success (e.g. egg production, hatching success or ATCase activity), and mortality rates (total and stage-specific) remain to be unraveled. These changing parameters may have effects for the zooplankton population dynamics, abundance, and on zooplankton-dependent trophic levels.

2. **Studies on temperature-driven higher energy requirements, feeding rates, and nutritional balance.** As described in the metabolic theory of ecology (Brown *et al.* 2004), higher temperatures increase the metabolism of organisms which leads to increased energy demands. Mesocosm experiments could show that copepod grazing had top down control on phytoplankton and ciliate biomass (Aberle *et al.* 2007; Sommer and Lewandowska 2011). Thus, understanding nutritional needs and the particular increased need of energy uptake are necessary to exclude food effects as drivers for body size and abundance changes.

3. **Further studies on multiple-stressor responses.** As described, global change does not act as single stressors on individuals or communities. Other stressors will also affect marine planktonic food webs. Especially for the Baltic Sea, the impact of hypoxia and nutrient availability are of interest as important stressors for planktonic organisms.

4. **Studies on thermal adaption of copepods.** Beside the observed phenological responses to global change, the adaptive response potential might be important to the entire ecosystem. Zooplankton are excellent model organisms for studies of the responses of animals to global change, because of the relatively short generation times (weeks to months), making them amenable to rapid evolutionary change. A trade-off arises when genotypes cannot have maximal fitness in two environments (Fry 2003). Organisms adapt to stress by either avoiding (resistance) or dealing with (tolerance) the negative effects of stress (Råberg *et al.* 2009). Both mechanisms involve expenditures for the organism and thus can incur fitness costs. It may be possible to discern the relative roles of ecological and evolutionary processes in determining the phenology and life history traits of populations (Gienapp *et al.* 2008). Additional assessment of the genetic basis and evolutionary potential of phenology and life history responses to global change on the individual level might help to understand the impacts and potential adaptation.

ACKNOWLEDGEMENTS

A number of people participated in the projects I was involved in and helped during the time of my experiments. Thus, I would like to thank these people, without whom my work would not have been possible and this thesis could not have emerged.

I am deeply grateful to my first supervisor Ulrich Sommer, who has always supported me during my work. I thank him for giving me the opportunity to start as a PhD candidate without any fixed funding. I thank him for sending me around the world to expand my knowledge, for developing ideas for my work, for his patience, all discussions and concrete replies to my questions.

I would like to express my heartfelt gratitude to my second supervisor Stefanie Ismar. It has been an honour to be her first PhD student. I could not have asked for a nicer and more available person to introduce me to scientific writing, hypothesis testing and “proper” statistics. I appreciate her contribution of time (and coffee) that we spend together to read through reviewer comments and corrections.

Maarten Boersma, Karen Wiltshire and Arne Malzahn from the Alfred-Wegener Institute are acknowledged for giving me the opportunity to conduct an experiment at “Biologische Anstalt Helgoland”. They helped me during this experiment with their knowledge, by asking the right questions and their experience. Especially, I want to thank Maarten Boersma for his patience during the hard process of writing chapter 2 and the related manuscript.

I thank Mary O’Connor for hosting me as visiting scientist, for helping me to apply for the mobility award, and especially for the nice and fruitful discussions during the experimental design, the experiment itself, and all her positive feedback about my work. The joy and enthusiasm she has for her research was contagious and motivational for me. I am also thankful for the excellent example she has set by successfully managing different projects and her personal life simultaneously.

I want to thank Anna Bockelmann und Barabara Santer for the nice atmosphere in our office, for lending their ears to my problems and especially for their motivational words in rough times. It was a pleasure to be your office-mate.

Jamileh Javidpour, Kalista H. Peter, and Carolin Paul are acknowledged for helping during the big mesocosm experiments and going through difficult and exhausting times during our experiments.

The EÖ-N working group has been a source of good advice and collaboration. Special thanks go to Thomas Hansen, Cordula Meyer, and Bente Gardeler for their technical support, highly precise measurements, and their help during times that I was overseas. Our secretary Gaby Barth I would like to thank for all administrative work she did to make my life easier.

I want to thank Carmen Arndt and Leni Hauss for discussing my scientific problems with me, their good remarks, sharing helpful literature on fatty acid and RNA/DNA analyses, and giving me feedback on my work.

I also have to mention my secret English tutor Ashlie Cipriano. She was always available for correcting my manuscripts.

Grateful acknowledgements are dedicated to all the hidden people in my private life for their patience, motivation and positive energy, which I needed very much to finish my thesis. I would like to thank you for all the inconvenient support and solutions to problems, which I also needed during rough times but also for celebrating reached milestones with me.

I want to address a big THANK YOU! to my family for their encouragement and trust.

REFERENCES

Aberle, N. *et al.* 2007. Spring bloom succession, grazing impact and herbivore selectivity of ciliate communities in response to winter warming. - Oecologia 150: 668-681.

Allen, A. P. *et al.* 2005. Linking the global carbon cycle to individual metabolism. - Funct. Ecol. 19: 202-213.

Arendt, J. 2007. Ecological correlates of body size in relation to cell size and cell number: Patterns in flies, fish, fruits and foliage. - Biol. Rev. 82: 241-256.

Arndt, C. and Sommer, U. 2014. Effect of algal species and concentration on development and fatty acid composition of two harpacticoid copepods, *Tisbe* sp. and *Tachidius discipes*, and a discussion about their suitability for marine fish larvae. - Aquacult. Nutr. 20: 44-59.

Arrhenius, S. 1889. Über die Reaktionsgeschwindigkeit bei Inversion von Rohrzucker durch Säuren. Zeitschrift für Physik Chemique, pp. 226-248.

Atkinson, A. 1996. Subantarctic copepods in an oceanic, low chlorophyll environment: ciliate predation, food selectivity and impact on prey populations. - Mar. Ecol. Prog. Ser. 130: 85-96.

Atkinson, D. 1994. Temperature and organism size - a biological law for ectotherms Advances in ecological research, vol 25, pp. 1-58.

Atkinson, D. 1995. Effects of temperature on the size of aquatic ectotherms - exceptions to the general rule. - J. Therm. Biol. 20: 61-74.

Atkinson, D. *et al.* 2003. Protists decrease in size linearly with temperature: ca. 2.5% degrees C^{-1}. - Proc. R. Soc. B Biol. Sci. 270: 2605-2611.

Atkinson, D. and Sibly, R. M. 1997. Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. - Trends Ecol. Evol. 12: 235-239.

Banse, K. 1995. Zooplankton: Pivotal role in the control of ocean production: I. Biomass and production. - ICES J. Mar. Sci. 52: 265-277.

Batten, S. D. and Walne, A. W. 2011. Variability in northwards extension of warm water copepods in the NE Pacific. - J. Plankton Res. 33: 1643-1653.

Beaugrand, G. *et al.* 2002. Reorganization of north atlantic marine copepod biodiversity and climate. - Science 296: 1692-1694.

Behrends, G. 1996. Long-term investigation of seasonal zooplankton dynamics in Kiel Bight, Germany. - Proc. 13th Baltic Mar. Biol. Symp.: 93-99.

Behrenfeld, M. J. *et al.* 2006. Climate-driven trends in contemporary ocean productivity. - Nature 444: 752-755.

Berberovic, R. 1990. Elemental composition of 2 coexisting daphnia species during the seasonal course of population development in lake constance. - Oecologia 84: 340-350.

Bergmann, K. 1847. Ueber die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse.

Blackburn, T. M. *et al.* 1999. Geographic gradients in body size: A clarification of Bergmann's rule. - Divers. Distrib. 5: 165-174.

Bochdansky, A. B. *et al.* 2005. Experimental evidence for selection against fish larvae with high metabolic rates in a food limited environment. - Mar. Biol. 147: 1413-1417.

Boersma, M. 2000. The nutritional quality of P-limited algae for *Daphnia*. - Limnol. Oceanogr. 45: 1157-1161.

Boersma, M. *et al.* 2008. Nutritional limitation travels up the food chain. - Int. Rev. Hydrobiol. 93: 479-488.

Boersma, M. *et al.* 2001. Nutritional quality of seston for the freshwater herbivore *Daphnia galeata x hyalina*: Biochemical versus mineral limitations. - Oecologia 129: 342-348.

Boltzmann, L. 1872. Weiter Studien über das Wärmegleichgewicht unter Gasmolekülen. Sitzungsberichte der Mathematisch-Naturwissenschaftlichen Classe der kaiserlichen Akademie der Wissenschaften Wien. pp. 275-370.

Bopp, L. *et al.* 2005. Response of diatoms distribution to global warming and potential implications: a global model study. - Geophys. Res. Lett. 32: L19606.

Boyce, D. G. *et al.* 2010. Global phytoplankton decline over the past century. - Nature 466: 591-596.

Breteler, W. C. M. K. *et al.* 2004. Role of essential lipids in copepod nutrition: No evidence for trophic upgrading of food quality by a marine ciliate. - Mar. Ecol. Prog. Ser. 274: 199-208.

Breteler, W. C. M. K. *et al.* 2005. Effect of diatom nutrient limitation on copepod development: role of essential lipids. - Mar. Ecol. Prog. Ser. 291: 125-133.

Brett, M. T. and Müller-Navarra, D. C. 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. - Freshwater Biol. 38: 483-499.

Briceño, H. O. and Boyer, J. N. 2010. Climatic controls on phytoplankton biomass in a sub-tropical estuary, Florida Bay, USA. - Estuaries and Coasts 33: 541-553.

Brown, J. H. *et al.* 2004. Toward a metabolic theory of ecology. - Ecology 85: 1771-1789.

Burkhardt, S. *et al.* 1999. Effect of CO_2 concentration on c: N: P ratio in marine phytoplankton: A species comparison. - Limnol. Oceanogr. 44: 683-690.

Caldeira, K. and Wickett, M. E. 2003. Oceanography: Anthropogenic carbon and ocean pH. - Nature 425: 365.

Campbell, R. G. *et al.* 2001. Growth and development rates of the copepod calanus finmarchicus reared in the laboratory. - Mar. Ecol. Prog. Ser. 221: 161-183.

Carpenter, S. R. and Kitchell, J. F. 1996. The trophic cascade in lakes. - Cambridge University Press.

Carrillo, P. *et al.* 2001. Relationship between N:P ratio and growth rate during the life cycle of calanoid copepods: an in situ measurement. - J. Plankton Res. 23: 537-547.

Claireaux, G. and Lefrancois, C. 2007. Linking environmental variability and fish performance: integration through the concept of scope for activity. - Philos. Trans. R. Soc. Lond. B. Biol. Sci. 362: 2031-2041.

Cleland, E. E. *et al.* 2007. Shifting plant phenology in response to global change. - Trends Ecol. Evol. 22: 357-365.

Clemmesen, C. 1993. Improvements in the fluorimetric determination of the RNA and DNA content of the individual marine fish larvae. - Mar. Ecol. Prog. Ser. 100: 177-183.

Cottingham, K. L. *et al.* 2005. Knowing when to draw the line: designing more informative ecological experiments. - Front. Ecol. Environ. 3: 145-152.

Darchambeau, F. *et al.* 2003. How *Daphnia* copes with excess carbon in its food. - Oecologia 136: 336-346.

Daufresne, M. *et al.* 2009. Global warming benefits the small in aquatic ecosystems. - P. Natl. Acad. Sci. USA 106: 12788-12793.

DeMott, W. R. 2003. Implications of element deficits for zooplankton growth. - Hydrobiologia 491: 177-184.

DeMott, W. R. *et al.* 1998. Effects of phosphorus-deficient diets on the carbon and phosphorus balance of *Daphnia magna*. - Limnol. Oceanogr. 43: 1147-1161.

DeMott, W. R. and Tessier, A. J. 2002. Stoichiometric constraints vs. algal defenses: testing mechanisms of zooplankton food limitations. - Ecology 83: 3426-3433.

Dewar, R. C. *et al.* 1999. Acclimation of the respiration/photosynthesis ratio to temperature: insights from a model. - Glob. Chang. Biol. 5: 615-622.

Dickson, A. G. 1981. An exact definition of total alkalinity and a procedure for the estimation of alkalinity and total inorganic carbon from titration data. - Deep Sea Research Part A. Oceanographic Research Papers 28: 609-623.

Dickson, A. G. *et al.* 2003. Reference materials for oceanic CO_2 analysis: A method for the certification of total alkalinity. - Mar. Chem. 80: 185-197.

Dickson, A. G. and Millero, F. J. 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. - Deep Sea Research Part A. Oceanographic Research Papers 34: 1733-1743.

Dobberfuhl, D. R. and Elser, J. J. 2000. Elemental stoichiometry of lower food web components in arctic and temperate lakes. - J. Plankton Res. 22: 1341-1354.

Doney, S. C. 2006. Oceanography: Plankton in a warmer world. - Nature 444: 695-696.

Drinkwater, K. F. 2006. The regime shift of the 1920s and 1930s in the north atlantic. - Prog. Oceanogr. 68: 134-151.

Durant, J. M. *et al.* 2007. Climate and the match or mismatch between predator requirements and resource availability. - Climate Res. 33: 271-283.

Edenhofer, O. *et al.* 2014. IPCC, 2014: Climate change 2014: Mitigation of climate change. Contribution of working group iii to the fifth assessment report of the intergovernmental panel on climate change. - Cambridge University Press.

Edwards, M. and Richardson, A. J. 2004. Impact of climate change on marine pelagic phenology and trophic mismatch. - Nature 430: 881-884.

Elser, J. J. *et al.* 1996. Organism size, life history, and n:P stoichiometry. - Bioscience 46: 674-684.

Elser, J. J. *et al.* 2001. Nutrient limitation reduces food quality for zooplankton: *Daphnia* response to seston phosphorus enrichment. - Ecology 82: 898-903.

Elser, J. J. *et al.* 2000. The evolution of ecosystem processes: growth rate and elemental stoichiometry of a key herbivore in temperate and arctic habitats. - J. Evol. Biol. 13: 845-853.

Eppley, R. W. 1972. Temperature and phytoplankton growth in the sea. - Fish. B. 70: 1063-1085.

Escribano, R. and McLaren, I. A. 1992. Influence of food and temperature on lengths and weights of 2 marine copepods. - J. Exp. Mar. Biol. Ecol. 159: 77-88.

Folt, C. L. *et al.* 1999. Synergism and antagonism among multiple stressors. - Limnol. Oceanogr. 44: 864-877.

Forster, J. and Hirst, A. G. 2012. The Temperature-Size rule emerges from ontogenetic differences between growth and development rates. - Funct. Ecol. 26: 483-492.

Forster, J. *et al.* 2011. How do organisms change size with changing temperature? The importance of reproductive method and ontogenetic timing. - Funct. Ecol. 25: 1024-1031.

Forster, J. *et al.* 2012. Warming-induced reductions in body size are greater in aquatic than terrestrial species. - P. Natl. Acad. Sci. USA 109: 19310-19314.

Forster, J. *et al.* 2011. Growth and development rates have different thermal responses. - Am. Nat. 178: 668-678.

Forster, P. *et al.* 2007. IPCC: Changes in atmospheric constituents and in radiative forcing Chapter 2. - Climate Change 20 (7).

Franke, A. and Clemmesen, C. 2011. Effect of ocean acidification on early life stages of Atlantic herring (*Clupea harengus* L.). – Biogeosciences Disc. 8 (4): 7097-7126.

Frommel A. Y. *et al.* 2011. Severe tissue damage in Atlantic cod larvae under increasing ocean acidification. – Nature Clim. Change 2 (1): 42-46.

Frost, T. M. *et al.* 1999. Multiple stresses from a single agent: diverse responses to the experimental acidification of Little Rock Lake, Wisconsin. - Limnol. Oceanogr. 44: 784-794.

Fry, J. D. 2003. Detecting ecological trade-offs using selection experiments. - Ecology 84: 1672-1678.

Fukami, T. *et al.* 2005. Species divergence and trait convergence in experimental plant community assembly. - Ecol. Lett. 8: 1283-1290.

Gardner, J. L. *et al.* 2011. Declining body size: A third universal response to warming? - Trends Ecol. Evol. 26: 285-291.

Gasol, J. M. *et al.* 1997. Biomass distribution in marine planktonic communities. - Limnol. Oceanogr. 42: 1356-1363.

Gaudy, R. and Verriopoulos, G. 2004. Spatial and seasonal variations in size, body volume and body proportion (prosome : Urosome ratio) of the copepod acartia tonsa in a semi-closed ecosystem (berre lagoon, western mediterranean). - Hydrobiologia 513: 219-229.

Geider, R. J. and La Roche, J. 2002. Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. - Eur. J. Phycol. 37: 1-17.

Gienapp, P. *et al.* 2008. Climate change and evolution: Disentangling environmental and genetic responses. - Molecular Ecology 17: 167-178.

Gilbert, B. *et al.* 2014. A bioenergetic framework for the temperature dependence of trophic interactions. - Ecol. Lett. 17: 902-914.

Gismervik, I. *et al.* 2002. Micro- and mesozooplankton response to enhanced nutrient input - a mesocosm study. - Hydrobiologia 484: 75-87.

Goodman, R. E. *et al.* 2012. Avian body size changes and climate change: warming or increasing variability? - Glob. Chang. Biol. 18: 63-73.

Goss, L. B. and Bunting, D. L. 1980. Temperature effects on zooplankton respiration. - Comp. Biochem. Phys. B 66 A: 651-658.

Guillard, R. R. and Ryther, J. H. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana hustedt*, and *Detonula confervacea* (cleve) gran. - Can. J. Microbiol. 8: 229-239.

Hansen, J. *et al.* 2010. Global surface temperature change. - Rev. Geophys. 48: RG4004.

Hansen, J. *et al.* 2014. Global temperature update through 2013.

Hansen, J. *et al.* 2006. Global temperature change. - Proceedings of the National Academy of Sciences 103: 14288-14293.

Hansson, I. 1973. A new set of acidity constants for carbonic acid and boric acid in sea water. - Deep- Sea Res. 20: 461-478.

Harley, C. D. G. *et al.* 2006. The impacts of climate change in coastal marine systems. - Ecol. Lett. 9: 228-241.

Havenhand, J. N. 2012. How will ocean acidification affect baltic sea ecosystems? An assessment of plausible impacts on key functional groups. - Amibo 41: 637-644.

Heinle, D. R. and Flemer, D. A. 1975. Carbon requirements of a population of the estuarine copepod eurytemora affinis. - Mar. Biol. 31: 235-247.

Hillebrand, H. *et al.* 1999. Biovolume calculations for pelagic and benthic microalgae. - J. Phycol. 35: 403-424.

Hirst, A. G. and Kiorboe, T. 2002. Mortality of marine planktonic copepods: Global rates and patterns. - Mar. Ecol. Prog. Ser. 230: 195-209.

Holste, L. and Peck, M. A. 2005. The effects of temperature and salinity on egg production and hatching success of baltic acartia tonsa (Copepoda: Calanoida): a laboratory investigation. - Mar. Biol. 148: 1061-1070.

Houghton, J. T. 1996. Climate change 1995: The science of climate change. - Cambridge University Press, Cambridge.

Huntley, M. E. and Lopez, M. D. G. 1992. Temperature-dependent production of marine copepods: A global synthesis. - Am. Nat. 140: 201-242.

Ikeda, T. *et al.* 2000. Metabolism. - Academic Press.

Ismar, S. M. H. *et al.* 2008. Effect of food concentration and type of diet on *Acartia* survival and naupliar development. - Mar. Biol. 154: 335-343.

James, F. C. 1970. Geographic size variation in birds and its relationship to climate. - Ecology 51: 365-&.

Jonasdottir, S. H. *et al.* 2009. Assessing the role of food quality in the production and hatching of *Temora longicornis* eggs. - Mar. Ecol. Prog. Ser. 382: 139-150.

Kim, J. M. *et al.* 2006. The effect of seawater CO_2 concentration on growth of a natural phytoplankton assemblage in a controlled mesocosm experiment. - Limnol. Oceanogr. 51: 1629-1636.

Kiorboe, T. *et al.* 1985. Bioenergetics of the planktonic copepod acartia tonsa - relation between feeding, egg-production and respiration, and composition of specific dynamic action. - Mar. Ecol. Prog. Ser. 26: 85-97.

Klein Breteler, W. C. M. and Schogt, N. 1994. Development of acartia clausi (copepoda, calanoida) cultured at different conditions of temperature and food. - Hydrobiologia 292-293: 469-479 LA - English.

Klein Breteler, W. C. M. *et al.* 1990. On the role of food quality in grazing and development of life stages and genetic change of body size during cultivation of pelagic copepods. - J. Exp. Mar. Biol. Ecol. 135: 177-189.

Kobari, T. and Ikeda, T. 2001. Ontogenetic vertical migration and life cycle of *Neocalanus plumchrus* (Crustacea : Copepoda) in the oyashio region, with notes on regional variations in body sizes. - J. Plankton Res. 23: 287-302.

Kordas, R. L. *et al.* 2011. Community ecology in a warming world: The influence of temperature on interspecific interactions in marine systems. - J. Exp. Mar. Biol. Ecol. 400: 218-226.

Koski, M. *et al.* 1998. Effect of food quality on rate of growth and development of the pelagic copepod pseudocalanus elongatus (Copepoda, Calanoida). - Mar. Ecol. Prog. Ser. 170: 169-187.

Kratina, P. *et al.* 2012. Warming modifies trophic cascades and eutrophication in experimental freshwater communities. - Ecology 93: 1421-1430.

Krueger, C. and Tian, L. 2004. A comparison of the general linear mixed model and repeated measures anova using a dataset with multiple missing data points. - Biological Research For Nursing 6: 151-157.

Kurihara, H. *et al.* 2004. Effects of raised CO_2 concentration on the egg production rate and early development of two marine copepods (*Acartia steueri and Acartia erythraea*). - Mar. Pollut. Bull. 49: 721-727.

Landry, M. R. 1983. The development of marine calanoid copepods with comment on the isochronal rule. - Limnol. Oceanogr. 28: 614-624.

Lane, A. E. and Burris, J. E. 1981. Effects of environmental ph on the internal pH of *Chlorella pyrenoidosa*, *Scenedesmus quadricauda*, and *Euglena mutabilis*. - Plant Physiol. 68: 439-442.

Le Quéré, C. *et al.* 2012. The global carbon budget 1959–2011. - Earth System Science Data Discussusion 5: 1107-1157.

Le Quéré, C. *et al.* 2013. The global carbon budget 1959–2011. - Earth Syst. Sci. Data 5: 165-185.

Leandro, S. M. *et al.* 2006. Temperature-dependent development and somatic growth in two allopatric populations of *Acartia clausi* (Copepoda : Calanoida). - Mar. Ecol. Prog. Ser. 322: 189-197.

Lee, R. *et al.* 1971. Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. - Mar. Biol. 9: 99-108.

Lehman, J. T. and Sandgren, C. D. 1982. Phosphorus dynamics of the procaryotic nannoplankton in a michigan lake. - Limnol. Oceanogr. 27: 828-838.

Lewandowska, A. M. *et al.* 2014. Effects of sea surface warming on marine plankton. - Ecol. Lett. 17 (5): 614-623.

Lewandowska, A. M. *et al.* 2012. Responses of primary productivity to increased temperature and phytoplankton diversity. - J. Sea Res. 72: 87-93.

Lewandowska, A. M. and Sommer, U. 2010. Climate change and the spring bloom: A mesocosm study on the influence of light and temperature on phytoplankton and mesozooplankton. - Mar. Ecol.-Prog. Ser. 405: 101-111.

Livingstone, D. M. 2003. Impact of secular climate change on the thermal structure of a large temperate central European lake. - Climatic Change 57: 205-225.

Lopez-Urrutia, A. *et al.* 2006. Scaling the metabolic balance of the oceans. - Proceedings of the National Academy of Sciences of the United States of America 103: 8739-8744.

Mackas, D. L. *et al.* 2012. Changing zooplankton seasonality in a changing ocean: Comparing time series of zooplankton phenology. - Prog. Oceanogr. 97: 31-62.

Malzahn, A. M. *et al.* 2007. Nutrient limitation of primary producers affects planktivorous fish condition. - Limnol. Oceanogr. 52: 2062-2071.

Malzahn, A. M. and Boersma, M. 2012. Effects of poor food quality on copepod growth are dose dependent and non-reversible. - Oikos 121: 1408-1416.

Malzahn, A. M. *et al.* 2010. Differential effects of nutrient-limited primary production on primary, secondary or tertiary consumers. - Oecologia 162: 35-48.

Marzolf, E. R. *et al.* 1994. Improvements to the diurnal upstream–downstream dissolved oxygen change technique for determining whole-stream metabolism in small streams. - Can. J. Fish. Aquat. Sci. 51: 1591-1599.

Mauchline, J. *et al.* 1998. Advances in marine biology - the biology of calanoid copepods - Introduction. Advances in marine biology, vol 33: The biology of calanoid copepods, pp. 1-9.

McArdle, B. H. and Lawton, J. H. 1979. Effects of prey-size and predator-instar on the predation of *Daphnia* by *Notonecta*. - Ecol. Entomol. 4: 267-275.

Mehrbach, C. *et al.* 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at the atmospheric pressure. - Limnol. Oceanogr. 18: 897-907.

Melzner, F. *et al.* 2013. Future ocean acidification will be amplified by hypoxia in coastal habitats. - Mar. Biol. 160: 1875-1888.

Menden-Deuer, S. and Lessard, E. J. 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. - Limnol. Oceanogr. 45: 569-579.

Millien, V. *et al.* 2006. Ecotypic variation in the context of global climate change: revisiting the rules. - Ecol. Lett. 9: 853-869.

Möllmann, C. *et al.* 2005. Climate, zooplankton, and pelagic fish growth in the Central Baltic Sea. - ICES J. Mar. Sci. 62: 1270-1280.

Möllmann, C. *et al.* 2008. Effects of climate and overfishing on zooplankton dynamics and ecosystem structure: regime shifts, trophic cascade, and feedback loops in a simple ecosystem. - ICES J. Mar. Sci. 65: 302-310.

Morán, X. A. G. *et al.* 2010. Increasing importance of small phytoplankton in a warmer ocean. - Glob. Chang. Biol. 16: 1137-1144.

Müller-Navarra, D. 1995. Evidence that a highly unsaturated fatty-acid limits *Daphnia* growth in nature. - Arch. Hydrobiol. 132: 297-307.

Müller-Navarra, D. C. *et al.* 2000. A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. - Nature 403: 74-77.

Müller, H. and Geller, W. 1993. Maximum growth rates of aquatic ciliated protozoa - the dependence on body size and temperature reconsidered. - Arch. Hydrobiol. 126: 315-327.

Müren, U. *et al.* 2005. Potential effects of elevated sea-water temperature on pelagic food webs. - Hydrobiologia 545: 153-166.

Norberg, J. and DeAngelis, D. 1997. Temperature effects on stocks and stability of a phytoplankton-zooplankton model and the dependence on light and nutrients. - Ecol. Model. 95: 75-86.

O'Connor, M. I. 2009. Warming strengthens an herbivore-plant interaction. - Ecology 90: 388-398.

O'Connor, M. I. *et al.* 2007. Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. - Proceedings of the National Academy of Sciences 104: 1266-1271.

O'Connor, M. I. *et al.* 2011. Theoretical predictions for how temperature affects the dynamics of interacting herbivores and plants. - The Amarican Naturalist 178: 626-638.

O'Connor, M. I. *et al.* 2009. Warming and resource availability shift food web structure and metabolism. - Plos Biology 7.

Orr, J. C. *et al.* 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. - Nature 437: 681-686.

Parmesan, C. and Yohe, G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. - Nature 421: 37-42.

Patridge, L. *et al.* 1994. Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. - Evolution 48: 1269-1276.

Pearman, P. B. *et al.* 2008. Prediction of plant species distributions across six millennia. - Ecol. Lett. 11: 357-369.

Persson, J. *et al.* 2011. Increased risk of phosphorus limitation at higher temperatures for *Daphnia magna*. - Oecologia 165: 123-129.

Petchey, O. L. *et al.* 1999. Environmental warming alters food-web structure and ecosystem function. - Nature 402: 69-72.

Peter, K. H. and Sommer, U. 2012. Phytoplankton cell size: Intra- and interspecific effects of warming and grazing. - PLoS one 7.

Pierrot, D. *et al.* 2006. CO2sys dos program developed for co2 system calculations. - ORNL/CDIAC-105. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, TN.

Plath, K. and Boersma, M. 2001. Mineral limitation of zooplankton: stoichiometric constraints and optimal foraging. - Ecology 82: 1260-1269.

Pörtner, H. 2008. Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. - Mar. Ecol. Prog. Ser. 373: 203-217.

Przeslawski, R. *et al.* 2008. Beyond corals and fish: The effects of climate change on noncoral benthic invertebrates of tropical reefs. - Glob. Chang. Biol. 14: 2773-2795.

Putt, M. and Stoecker, D. K. 1989. An experimentally determined carbon - volume ratio for marine oligotrichous ciliates from estuarine and coastal waters. - Limnol. Oceanogr. 34: 1097-1103.

Råberg, L. *et al.* 2009. Decomposing health: Tolerance and resistance to parasites in animals. - Philos. Trans. R. Soc. Lond. B. Biol. Sci. 364: 37-49.

Reading, C. J. 2007. Linking global warming to amphibian declines through its effects on female body condition and survivorship. - Oecologia 151: 125-131.

Rossoll, D. *et al.* 2012. Ocean acidification-induced food quality deterioration constrains trophic transfer. - PLoS one 7: e34737.

Rossoll, D. *et al.* 2013. Community interactions dampen acidification effects in a coastal plankton system. - Mar. Ecol. Prog. Ser. 486: 37-46.

Rothhaupt, K. O. 1995. Algal nutrient limitation affects rotifer growth rate but not ingestion rate. - Limnol. Oceanogr. 40: 1201-1208.

Saiz, E. *et al.* 1998. RNA content of copepods as a tool for determining adult growth rates in the field. - Limnol. Oceanogr. 43: 465-470.

Sanford, E. 1999. Regulation of keystone predation by small changes in ocean temperature. - Science 283: 2095-2097.

Sargent, J. R. *et al.* 1997. Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. - Aquaculture 155: 117-127.

Scheffer, M. *et al.* 2001. Catastrophic shifts in ecosystems. - Nature 413: 591-596.

Schiermeier, Q. 2006. Climate change: A sea change. - Nature 439: 256-260.

Schindler, D. E. *et al.* 1997. Influence of food web structure on carbon exchange between lakes and the atmosphere. - Science 277: 248-251.

Schindler, D. W. 2001. The cumulative effects of climate warming and other human stresses on canadian freshwaters in the new millennium. - Can. J. Fish. Aquat. Sci. 58: 18-29.

Schmittner, A. 2005. Decline of the marine ecosystem caused by a reduction in the atlantic overturning circulation. - Nature 434: 628-633.

Schoo, K. L. *et al.* 2009. Does the nutrient stoichiometry of primary producers affect the secondary consumer pleurobrachia pileus? - Aquat. Ecol. 44: 233-242.

Schoo, K. L. *et al.* 2014. The reaction of European lobster larvae (*Homarus gammarus*) to different quality food: effects of ontogenetic shifts and pre-feeding history. - Oecologia 174: 581-594.

Schoo, K. L. *et al.* 2013. Increased carbon dioxide availability alters phytoplankton stoichiometry and affects carbon cycling and growth of a marine planktonic herbivore. - Mar. Biol. 160: 2145-2155.

Shannon, C. E. and Weaver, W. 1963. Mathematical theory of communication. - University Illinois Press.

Sheridan, J. A. and Bickford, D. 2011. Shrinking body size as an ecological response to climate change. - Nat. Clim. Chang. 1: 401-406.

Shurin, J. B. *et al.* 2012. Warming shifts top-down and bottom-up control of pond food web structure and function. - Philos. Trans. R. Soc. Lond. B. Biol. Sci. 367: 3008-3017.

Siegenthaler, U. *et al.* 2005. Stable carbon cycle-climate relationship during the late pleistocene. - Science 310: 1313-1317.

Smol, J. P. *et al.* 2005. Climate-driven regime shifts in the biological communities of arctic lakes. - Proceedings of the National Academy of Sciences of the United States of America 102: 4397-4402.

Sommer, U. *et al.* 2007. An indoor mesocosm system to study the effect of climate change on the late winter and spring succession of baltic sea phyto- and zooplankton. - Oecologia 150: 655-667.

Sommer, U. *et al.* 2012. The response of temperate aquatic ecosystems to global warming: novel insights from a multidisciplinary project. - Mar. Biol. 159: 2367-2377.

Sommer, U. *et al.* 2012. Beyond the plankton ecology group (PEG) model: Mechanisms driving plankton succession. - Annu. Rev. Ecol. Evol. Syst. 43: 429-448.

Sommer, U. and Lengfellner, K. 2008. Climate change and the timing, magnitude, and composition of the phytoplankton spring bloom. - Glob. Chang. Biol. 14: 1199-1208.

Sommer, U. and Lewandowska, A. M. 2011. Climate change and the phytoplankton spring bloom: Warming and overwintering zooplankton have similar effects on phytoplankton. - Glob. Chang. Biol. 17: 154-162.

Sterner, R. W. and Elser, J. J. 2002. Ecological stochiometry: the biology of elements from molecules to the biosphere. - Princeton University Press.

Stibor, H. *et al.* 2004. Copepods act as a switch between alternative trophic cascades in marine pelagic food webs. - Ecol. Lett. 7: 321-328.

Stockwell, D. A. *et al.* 2001. Anomalous conditions in the south-eastern bering sea, 1997: Nutrients, phytoplankton and zooplankton. - Fisheries Oceanography 10: 99-116.

Sweeney, B. W. *et al.* 1986. The relative importance of temperature and diet to larval development and adult size of the winter stonefly, *Soyedina carolinensis* (Piecoptera: Nemouridae). - Freshwater Biol. 16: 39-48.

Taucher, J. *et al.* 2012. Enhanced carbon overconsumption in response to increasing temperatures during a mesocosm experiment. - Biogeosciences 9: 3531-3545.

Thomas, W. H. *et al.* 1963. Thermal gradient incubators for small aquatic organisms. - Limnol. Oceanogr. 8: 357-360.

Thomsen, J. *et al.* 2010. Calcifying invertebrates succeed in a naturally CO_2-rich coastal habitat but are threatened by high levels of future acidification. - Biogeosciences 7: 3879-3891.

Thresher, R. E. *et al.* 2007. Depth-mediated reversal of the effects of climate change on long-term growth rates of exploited marine fish. - Proceedings of the National Academy of Sciences of the United States of America 104: 7461-7465.

Todd, C. D. *et al.* 2008. Detrimental effects of recent ocean surface warming on growth condition of Atlantic salmon. – Blobal Change Biology 14 (5): 958-970.

Townsend, C. R. *et al.* 2008. Individual and combined responses of stream ecosystems to multiple stressors. - Journal of Applied Ecology 45: 1810-1819.

Utermöhl, H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. - Mitt. int. Ver. theor. angew. Limnol. 9: 1-38.

van der Have, T. M. and de Jong, G. 1996. Adult size in ectotherms: temperature effects on growth and differentiation. - J. Theor. Biol. 183: 329-340.

Vasseur, D. A. and McCann, K. S. 2005. A mechanistic approach for modeling temperature-dependent consumer-resource dynamics. - Am. Nat. 166: 184-198.

Verity, P. G. and Smetacek, V. 1996. Organism life cycles, predation, and the structure of marine pelagic ecosystems. - Mar. Ecol. Prog. Ser. 130.

Vijverberg, J. and Frank , T. H. 1976. The chemical composition and energy contents of copepods and cladocerans in relation to their size. - Freshwater Biol. 6: 333-345.

Villegas, C. T. and Kanazawa, A. 1979. Relationship between diet composition and growth rate of the zoel and mysis stages of *Penaeus japonicus* bate. - Fish. Res. J. Philip. 4: 32-40.

Vinebrooke, R. D. *et al.* 2004. Impacts of multiple stressors on biodiversity and ecosystem functioning: The role of species co-tolerance. - Oikos 104: 451-457.

Vrede, T. *et al.* 1999. Phosphorus distribution in three crustacean zooplankton species. - Limnol. Oceanogr. 44: 225-229.

Walther, G. R. *et al.* 2002. Ecological responses to recent climate change. - Nature 416: 389-395.

Wiltshire, K. H. *et al.* 2008. Resilience of north sea phytoplankton spring blooms dynamics: an analysis of long term data at helgoland roads. - Limnol. Oceanogr. 53: 1294-1302.

Winder, M. and Sommer, U. 2012. Phytoplankton response to a changing climate. - Hydrobiologia 698: 5-16.

Wohlers, J. *et al.* 2009. Changes in biogenic carbon flow in response to sea surface warming. - Proceedings of the National Academy of Sciences of the United States of America 106: 7067-7072.

Woodwell, G. M. *et al.* 1998. Biotic feedbacks in the warming of the earth. - Climatic Change 40: 495-518.

Yom-Tov, Y. and Geffen, E. 2011. Recent spatial and temporal changes in body size of terrestrial vertebrates: probable causes and pitfalls. - Biol. Rev. 86: 531-541.

Yom-Tov, Y. *et al.* 2010. Recent changes in body size of the eurasian otter *Lutra lutra* in Sweden. - Amibo 39: 496-503.

Young, T. P. *et al.* 2001. Community succession and assembly: comparing, contrasting and combining paradigms in the context of ecological restoration. - Ecological Restoration 19: 5-18.

Yvon-Durocher, G. and Allen, A. P. 2012. Linking community size structure and ecosystem functioning using metabolic theory. - Philos. Trans. R. Soc. Lond. B. Biol. Sci. 367: 2998-3007.

Yvon-Durocher, G. *et al.* 2012. Reconciling the temperature dependence of respiration across timescales and ecosystem types. - Nature advance online publication.

Yvon-Durocher, G. *et al.* 2010. Warming alters the metabolic balance of ecosystems. - Philos. Trans. R. Soc. Lond. B. Biol. Sci. 365: 2117-2126.

APPENDIX

Tab. 1.1 *Acartia* sp. stage duration (d) estimated at 3 temperatures (°C) and under saturating food conditions (≥1000 µg C l^{-1}). Mean value (±SE), n=2 (according to Leandro *et al.* 2006).

stage	10°C	15°C	18°C
N1	2.0 ±0.39	1.5±0.2	0.7±0.04
N2	5.9±0.71	3.2±0.35	1.9±0.01
N3	2.6±0.4	2.0±0.31	1.1±0.17
N4	2.2±0.12	1.5±0.40	0.9±0.16
N5	2.2±0.5	1.9±0.03	1.2±0.02
N6	2.4±0.15	2.3±0.37	0.8±0.05
C1	2.3±0.39	2.2	1.2±0.02
C2	3.5±0.13	3.0	1.1±0.05
C3	3.3±	2.4	1.4±0.06
C4	3.8	2.8	1.5±0.01
C5	4.7	4.5	2.8±0.17

Tab. 1.2 Correlation of phytoplankton and ciliate biomass during experimental period (ANOVA: P=0.452, dF=2, F-value=0.91).

temperature	mesocosm	Correlation coefficient
9.5 °C	4	-0.7270
9.5 °C	5	-0.9188
9.5 °C	6	-0.6356
13.5 °C	7	-0.7364
13.5 °C	8	-0.3852
13.5 °C	9	-0.6501
17.5 °C	10	-0.6111
17.5 °C	11	-0.6808
17.5 °C	12	-0.9941

Tab. 1.3 ANOVA results temperature effect on phytoplankton and ciliate correlation coefficients during experimental period.

Term	Sum Squares	Mean Squares	F-value	p-value	dF
temperature	0.0583	0.0291	0.9103	0.452	2
Residuals	0.1920	0.0320			6

Tab. 1.4 Correlation coefficients of adult *Acartia* sp. and total phytoplankton biomass of last experimental day.

temperature	**Correlation coefficient**
9.5	-0.994
13.5	0.949
17.5	0.970

Tab. 1.5 ANOVA results of temperature effects on correlation coefficients of adult *Acartia* sp. and phytoplankton biomass (R^2=0.163).

Factor	dF	Sum. Squares	Mean Squares	F-value	p-value
temperature	2	0.1835	0.0917	0.5833	0.587
Residuals	6	0.9437	0.1573		

Tab. 1.6 Absolute and relative prosome length changes of *Acartia* sp. to mean size of ambient temperature treatment

Acartia sp.	C1	C2	C3	C4	C5	adult
Warming 13.5°C to 17.5 °C [Mean size (SD)]	/	/	10.58 µm (-2.60%) [406.63µm (3.15)]	5.07 µm (-1.06%) [476.92µm (7.56)]	4.18 µm (+ 0.73%) [574.06µm (4.51)]	65.19 µm (-9.69%) [672.67µm (32.72)]
Cooling 13.5°C to 9.5 °C [Mean size (SD)]	12.60 µm (+4.48%) [281.3µm (3.39)]	9.07 µm (+2.60%) [348.17µm (0.45)]	35.27 µm (+8.67%) [406.63µm (3.15)]	54.99 µm (+11.53%) [476.92µm (7.56)]	68.57 µm (+11.94%) [574.03µm (4.51)]	57.96 µm (+8.62%) [672.67µm (32.72)]

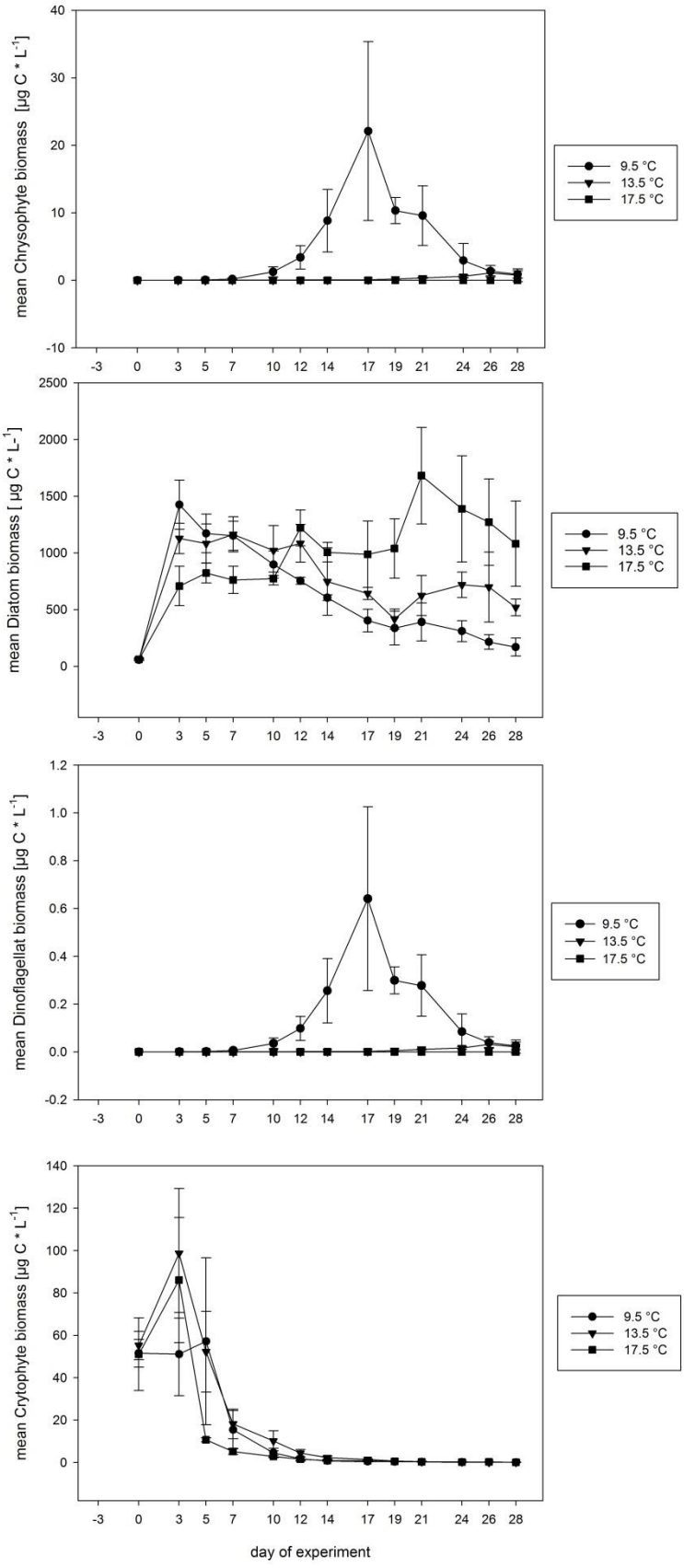


Fig. 1.1 Edible phytoplankton species biomasses over the course of the experiment

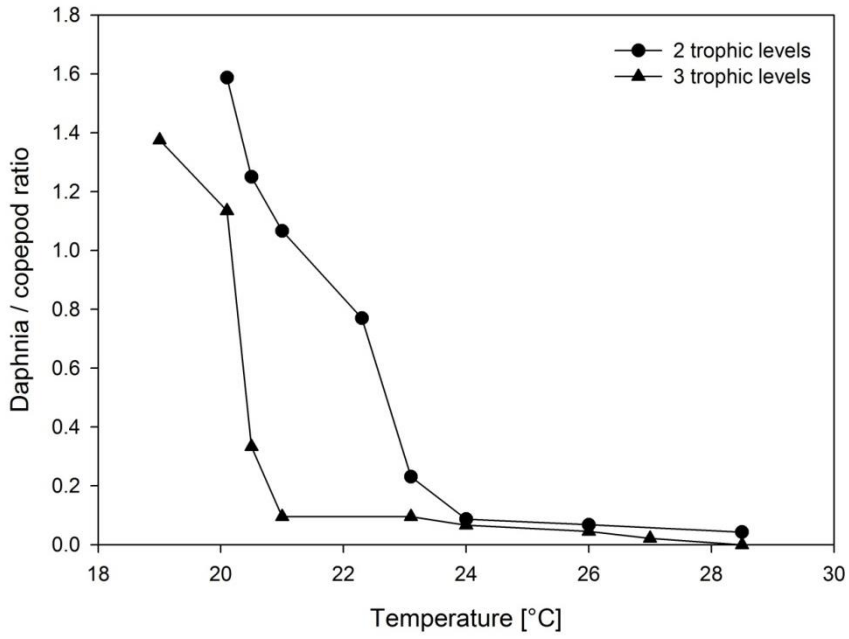


Fig. 4.1 *Daphnia*/copepod abundance ratio of week 8.

CURRICULUM VITAE

Name:	Jessica Garzke
Geburtsdatum:	13. Juni 1983
Geburtsort:	Bergisch Gladbach
Staatsangehörigkeit:	deutsch

Werdegang

Seit Juni 2010	Promotion am Geomar Helmholtz-Zentrum für Ozeanforschung Kiel, Kiel, Deutschland
Okt 2006 – Juni 2010	Diplom Biologie Christian-Albrechts-Universität zu Kiel, Kiel, Deutschland
Okt 2003 – Okt 2006	Vordiplom in Biologie Christian-Albrechts-Universität zu Kiel, Kiel, Deutschland
Aug 1994 – Juni 2003	Gymnasium Herkenrath, Nordrhein-Westfalen, Deutschland Abitur

SCIENTIFIC CONTRIBUTION

Description of the individual scientific contribution to the multiple-author papers

The chapters of this thesis are partly accepted (chapter 1), ready for submission (chapter 2), under review (chapter 3), or in preparation (chapter 4) for submission to a scientific journal. This list serves as a clarification of my personal contribution to each publication

Chapter 1:
Climate change affects low trophic level marine consumers: warming decreases copepod size and abundance
Authors: Jessica Garzke, Stefanie M. H. Ismar and Ulrich Sommer;
Published in Oecologia (accepted) doi: 10.1007/s00442-014-3130-4
Contributions: JG and US designed the experiment; JG performed the experiment; JG, SMHI analyzed the data and wrote the manuscript; US provided editorial advice.

Chapter 2:
Interacting effects of nutrient limitation and temperature on size and growth rate of the copepod *Acartia tonsa*
Authors: Jessica Garzke, Arne M. Malzahn, Karen H. Wiltshire, Ulrich Sommer and Maarten Boersma
Ready for submisson
Contributions: JG, AMM, MB, KHW and US conceived research; JG and AMM performed the experiment; JG analysed the data; JG, AMM and MB discussed the results; JG wrote the manuscript.

Chapter 3:
Multi-stressor impacts of ocean warming and acidification on copepod abundance, body size and fatty acid content
Authors: Jessica Garzke, Thomas Hansen, Stefanie M.H. Ismar and Ulrich Sommer
Under review
Contributions: JG, SMHI and US conceived research; JG performed the experiment; TH analysed carbon chemistry; JG and SMHI discussed results; JG analysed the data; JG wrote the manuscript; US and SMHI provided editorial advice.

Chapter 4:

Temperature-driven declines in plankton biomass increase mass-specific ecosystem fluxes: higher trophic levels compensate for temperature driven metabolic changes

Authors: Jessica Garzke, Mary I. O'Connor and Ulrich Sommer

In preparation

Contributions: JG, MIO and US conceived research; JG performed experiment; JG and MIO discussed results; JG analysed the data; JG wrote the manuscript; MIO and US provided editorial advice.

ERKLÄRUNG

Hiermit erkläre ich, dass die vorliegende Dissertation, abgesehen von der Beratung meiner Betreuer, selbständig von mir angefertigt wurde und dass sie nach Form und Inhalt meine eigene Arbeit ist. Sie wurde keiner anderen Stelle im Rahmen einer Prüfungsverfahrens vorgelegt. Dies ist mein einziges und bisher erstes Promotionsverfahren. Die Promotion soll im Fach *Biological Oceanography* erfolgen. Des Weiteren erkläre ich, dass ich Zuhörer bei der Disputation zulasse.