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The response of three dominant Arctic copepod species to elevated CO_2 concentrations and water temperatures

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Meiner Familie

CONTENTS

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AWI	Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research
BIOACID	Biological Impacts of Ocean Acidification
C	Carbon
CCS	Carbon capture and storage
Chl *a*	Chlorophyll *a*
CI - CV	Copepodite stage I - V
CO_2	Carbon dioxide
CO_3^{2-}	Carbonate ion
DIC	Dissolved inorganic carbon
EPR	Egg production rate
GEOMAR	GEOMAR, Helmholtz Centre for Ocean Research Kiel
GS	Gonad development stage
H^+	Hydrogen ion
HCO_3^-	Bicarbonate ion
H_2CO_3	Carbonic acid
H_2O	Water
HPTS	8-Hydroxypyrene-1,3,6-trisulfonic acid, trisodium salt
ind.	Individual
KOSMOS	Kiel Off-Shore Mesocosms for Future Ocean Simulations
µatm	Microatmosphere
N	Nitrogen
Na_2SO_3	Sodium sulfite
NI - NVI	Nauplius stage I - VI
O_2	Oxygen
OA	Ocean acidification
pCO_2	Carbon dioxide partial pressure
pH_e	Extracellular pH
ppm	Parts per million
RV	Research vessel
S	Senescent females
SOPRAN	Surface Ocean Processes in the Anthropocene
spp.	Species

SUMMARY

Ocean acidification (OA), i.e. the uptake of man-made CO_2 and the subsequent decline in seawater pH, and ocean warming have the potential to severely affect the performance of marine organisms, their trophic interactions and, finally, whole ecosystems. In Arctic waters, the calanoid copepod species *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* are key components of the lipid-based food web, linking primary production and higher trophic levels. Within the framework of the research project BIOACID (Biological Impacts of Ocean Acidification), this study aims to provide a comprehensive overview on the sensitivity of the three *Calanus* species to ocean acidification and ocean warming. In controlled laboratory CO_2 incubation experiments, direct physiological and ecological effects of elevated pCO_2 were investigated in both active and diapausing *Calanus* life stages, and synergistic effects of pCO_2 and temperature were studied. In addition, indirect effects of OA via altered food regimes were tackled in an ecosystem-scale experiment within the framework of the SOPRAN (Surface Ocean Processes in the Anthropocene) mesocosm study in 2011.

Late copepodites and adult females of *C. finmarchicus*, *C. glacialis* and *C. hyperboreus* have been proven to be rather robust to elevated seawater pCO_2 in terms of direct effects. In active life stages, metabolic rates and thus the energetic demand were unaffected by CO_2 concentrations of up to 3000 µatm (pH 7.2), which is well beyond the levels projected to occur in surface waters during the next three centuries. Accordingly, food uptake and body mass were not altered, suggesting that also the available energy for processes such as reproduction and growth will not directly be affected by OA in future decades.

During their resting phase in deep waters in fall/winter, copepods might be especially threatened by climate change induced stress as they are not able to compensate for elevated energetic needs. However, our long-term incubation experiments over 17 weeks did not indicate that elevated pCO_2 affects diapausing *Calanus*, as neither respiration rates, body mass, gonad development nor the acid-base status in the hemolymph were altered in *C. hyperboreus* females, which were incubated at high CO_2 conditions compared to the control. The extracellular pH was found to be extremely low (< 6.6) in diapausing *Calanus*. This suggests that diapausing copepods may not be

challenged by the threats of elevated seawater pCO_2 due to their ability of regulating their acid-base status.

Significant effects were, however, found when copepods were exposed to elevated pCO_2 in combination with higher seawater temperatures. At 3000 µatm CO_2 and 5 °C, *C. hyperboreus* females lost significantly more body mass as compared to copepods kept at control conditions, indicating that they suffered from elevated energetic demands. However, respiration, gonad maturation, extracellular pH and survival were not affected.

Elevated temperatures (5 and 10 °C) in general had greater effects on the copepods than CO_2, altering respiration, gonad development and probably decreasing the total reproductive output. However, a climate change-induced temperature increase of up to 10 °C will unlikely occur in nature, and it remains open whether more realistic temperature changes will still affect the performance of diapausing *Calanus* species.

Indirect effects of OA via altered food regimes might be a more serious threat to the Arctic *Calanus* species as compared to direct effects. During the mesocosm study, indications were found that the copepod's body mass might be negatively affected in high pCO_2 environments. OA has the potential to alter the size regime of the phytoplankton community, favoring the growth of picoplankton. As the Arctic *Calanus* species are not able to successfully graze on these small organisms, they might become severely affected in future decades. Thus, future studies should more extensively focus on the indirect effects of OA on *Calanus* species and the copepod community in general, also in combination with elevated temperatures.

ZUSAMMENFASSUNG

Anthropogene CO_2-Emissionen führen zu einem stetig ansteigenden CO_2-Gehalt sowie steigenden Temperaturen sowohl in der Atmosphäre als auch in den Ozeanen. Durch die Aufnahme von CO_2 sinkt der pH-Wert des Meerwassers. Diese „Ozeanversauerung" kann marine Lebewesen in vielerlei Hinsicht negativ beeinflussen, was Auswirkungen auf die trophischen Interaktionen sowie letztendlich auch auf das gesamte Ökosystem haben kann. Die calanoiden Copepoden *Calanus finmarchicus*, *C. glacialis* und *C. hyperboreus* sind Schlüsselarten im Nahrungsnetz arktischer Gewässer und stellen ein Bindeglied zwischen der Primärproduktion und höheren trophischen Ebenen dar. Innerhalb des Verbundprojektes BIOACID (Biological Impacts of Ocean Acidification) war es Ziel dieser Arbeit, einen umfassenden Überblick über die Sensitivität der drei *Calanus*-Arten gegenüber der Ozeanversauerung und -erwärmung zu erhalten. In kontrollierten Laborexperimenten wurde untersucht, inwieweit aktive und überwinternde *Calanus*-Individuen direkt durch erhöhte CO_2-Konzentrationen beeinflusst werden. Zudem wurde getestet, ob eine gleichzeitige Erhöhung von CO_2-Gehalt und Temperatur synergistische Effekte in den Copepoden auslöst. Hierzu wurden sowohl physiologische als auch ökologische Parameter untersucht. Darüber hinaus wurde im Rahmen der SOPRAN (Surface Ocean Processes in the Anthropocene) Mesokosmen-Studie 2011 untersucht, ob Ozeanversauerung *Calanus*-Arten auf indirekte Weise, also durch Änderungen im Nahrungsangebot, beeinflusst.

Subadulte und adulte Lebensstadien von *C. finmarchicus*, *C. glacialis* und *C. hyperboreus* scheinen relativ unempfindlich gegenüber erhöhten CO_2-Konzentrationen im Seewasser zu sein. Ein pCO_2 von 3000 µatm (pH 7.2), welcher die vorhergesagten CO_2-Konzentrationen für die nächsten 300 Jahre übersteigt, hatte keine negative Wirkung auf die Respiration und damit den Energiebedarf der arktischen *Calanus*-Arten während ihrer aktiven Phase im Frühling und Sommer. Auch die Nahrungsaufnahme und das Körpergewicht der Copepoden änderten sich nicht, so dass die den Copepoden zur Verfügung stehende Energie, die z.B. für Reproduktion oder Wachstum benötigt wird, wahrscheinlich nicht direkt durch Ozeanversauerung beeinflusst wird.

Während der Diapause im Herbst und Winter, die die *Calanus*-Arten im Tiefenwasser verbringen, stellt durch veränderte Umweltbedingungen ausgelöster Stress ein erhebliches Risiko für die Tiere dar, da ein erhöhter Energiebedarf während dieser Zeit

nicht durch eine erhöhte Nahrungsaufnahme ausgeglichen werden kann. Unsere Langzeit-Inkubationen über 17 Wochen haben jedoch gezeigt, dass weder Atmung noch Körpergewicht, Gonadenentwicklung oder der Säure-Base-Haushalt in der Hämolymphe der Copepoden während des Ruhestadiums durch einen erhöhten pCO_2 beeinflusst wird. Der extrazelluläre pH der Tiere war während der Diapause extrem niedrig (< 6.6). Durch diese Fähigkeit, den Säure-Base-Haushalt erheblich regulieren zu können, scheinen die Copepoden keine Mühe zu haben, mit erhöhten CO_2-Konzentrationen im Meerwasser umzugehen.

Eine gleichzeitige Erhöhung von CO_2-Gehalt und Temperatur hatte dagegen einen signifikanten Einfluss auf *C. hyperboreus.* Weibchen, die bei 3000 µatm CO_2 und 5 °C inkubiert wurden, waren nach 17 Wochen signifikant leichter als Weibchen aus Kontrollbedingungen, was auf einen erhöhten Energiebedarf unter Ozeanversauerung und -erwärmung schließen lässt. Respirationsraten, Gonadenreife, der extrazelluläre pH und die Mortalität waren allerdings nicht beeinflusst.

Erhöhte Temperaturen (5 und 10 °C) hatten im Allgemeinen einen größeren Effekt auf *Calanus* als die Ozeanversauerung. Hier waren die Respirationsraten, die Gonadenentwicklung und evtl. auch die gesamte Reproduktionsleistung signifikant beeinträchtigt. Da die *Calanus*-Arten in der Natur, insbesondere während der Diapause, keinen solch hohen Temperaturen wie im Versuch ausgesetzt sein werden, bleibt es offen, ob auch realistischere Temperaturänderungen einen Effekt auf die Copepoden haben werden.

Indirekte Effekte der Ozeanversauerung, die durch Änderungen im Nahrungssystem ausgelöst werden, scheinen eine größere Bedrohung für die arktischen *Calanus*-Arten darzustellen als direkte Effekte. Während eines Mesokosmen-Experimentes wurden Anzeichen dafür gefunden, dass das Körpergewicht von Copepoden, die unter hohen CO_2-Bedingungen gehalten wurden, beeinträchtigt sein kann. Ozeanversauerung kann zu Änderungen im Größenspektrum der Phytoplankton-Gemeinschaft führen, wobei die Entwicklung von Picoplankton begünstigt zu sein scheint. Da die arktischen *Calanus*-Arten die kleinen Picoplankton-Organismen nicht verwerten können, könnte ihre Entwicklung dadurch stark beeinträchtigt werden. Dies sollte in zukünftigen Studien, auch in Kombination mit erhöhten Temperaturen, ausführlicher untersucht werden.

1 GENERAL INTRODUCTION

1.1 Anthropogenic climate change

Since the beginning of the industrial revolution in the middle of the 18th century, more than 2000 gigatonnes of carbon dioxide (CO_2) have been released by human activities, i.e. the burning of fossil fuels, cement manufactory and land-use changes (Le Quéré et al. 2013). Subsequently, the CO_2 concentration in the atmosphere increased from a preindustrial value of about 280 ppm (Solomon et al. 2007) to 395 ppm in 2013 (Dlugokencky and Tans 2013). This current CO_2 concentration and probably also the rate of change are unprecedented for the past 800,000 years (Stocker et al. 2013). As CO_2 is a greenhouse gas, its increasing concentration in the atmosphere changes the radiative forcing. Therefore, the global mean surface temperatures have already increased by 0.61 °C between 1850-1900 and 1986-2005 (Stocker et al. 2013). Subsequently, also the world's oceans become warmer, not only at the surface, where temperatures increased by 0.4 to 0.8 °C since the late 19th century (Albritton et al. 2001), but also in the deep sea (Soltwedel et al. 2005, Somavilla et al. 2013).

1.1.1 Ocean acidification - the other CO_2 problem

The CO_2 concentration in the surface waters of the world's oceans stays more or less at equilibrium with that in the atmosphere, which makes the oceans an important carbon sink. They have absorbed more than one fourth of the carbon emitted by human activities so far (Stocker et al. 2013) and therefore mitigate global warming. Without this process, atmospheric CO_2 concentrations would be approximately 450 ppm today (Doney et al. 2009). However, the CO_2 uptake changes the chemistry of the seawater and makes it more acidic (Box 1, Fig. 1.1), a process commonly referred to as 'Ocean Acidification' (OA). Since preindustrial times, the average surface ocean pH already decreased by 0.1 units, which corresponds to a 26 % increase in hydrogen ion concentration (Stocker et al. 2013). Model calculations based on the "business-as-usual" scenario IS92a of the Intergovernmental Panel on Climate Change project atmospheric CO_2 concentrations of approximately 750 ppm around the year 2100 and more than 1900 ppm around 2300 (Caldeira and Wickett 2003). At such high CO_2 concentrations, the seawater pH at the ocean surface is projected to decrease by ~0.4 units until 2100 and by a maximum of ~0.77 around 2300 (Fig. 1.2, Caldeira and Wickett 2003).

Box 1 Carbon dioxide chemistry in seawater

In seawater, dissolved CO_2 occurs in three inorganic forms, i.e. aqueous CO_2 (including carbonic acid (H_2CO_3), ~1 %), bicarbonate ions (HCO_3^-, ~91 %) and carbonate ions (CO_3^{2-}, ~8%), which together form the pool of dissolved inorganic carbon (DIC). The relative proportion of these three forms of DIC controls the seawater pH (Raven et al. 2005, Fig. B1.1).

When CO_2 from the atmosphere dissolves in seawater, it reacts with water to form carbonic acid (equation 1), which splits up into hydrogen ions (H^+) and bicarbonate ions (equations 2).

$$[CO_2] + [H_2O] \rightarrow [H_2CO_3] \qquad \text{(Eq. 1)}$$

$$[H_2CO_3] \rightarrow [H^+] + [HCO_3^-] \qquad \text{(Eq. 2)}$$

The addition of $[H^+]$ leads to an acidification of the seawater (i.e. a decrease in pH). The DIC system acts to diminish such acidification, as carbonate ions react with part of the hydrogen ions, forming bicarbonate (the so-called 'carbonate buffer'; equation 3). The continuous uptake of CO_2 from the atmosphere, however, diminishes the buffer capacity of the seawater.

$$[H^+] + [CO_3^{2-}] \rightarrow [HCO_3^-] \qquad \text{(Eq. 3)}$$

In summary, the dissolution of atmospheric CO_2 in the oceans leads to increasing amounts of H^+, H_2CO_3 and HCO_3^-, while the concentration of CO_3^{2-} decreases (Raven et al. 2005).

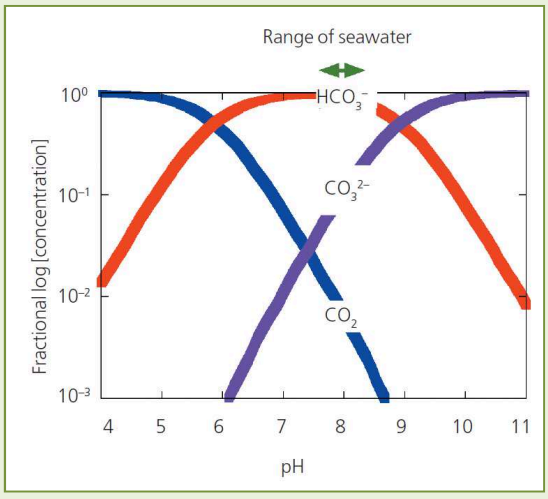

Fig. B1.1 Relative proportions of the three inorganic forms of CO_2 dissolved in seawater: aqueous CO_2 (including carbonic acid (H_2CO_3)), bicarbonate ions (HCO_3^-) and carbonate ions (CO_3^{2-}). The green arrow indicates the range of likely pH values in present and future oceans (from Raven et al. 2005).

As the solubility of CO_2 is higher in cold as compared to warm waters, most rapid changes in ocean chemistry will occur in high latitude areas (Orr et al. 2005, Fabry et al. 2009).

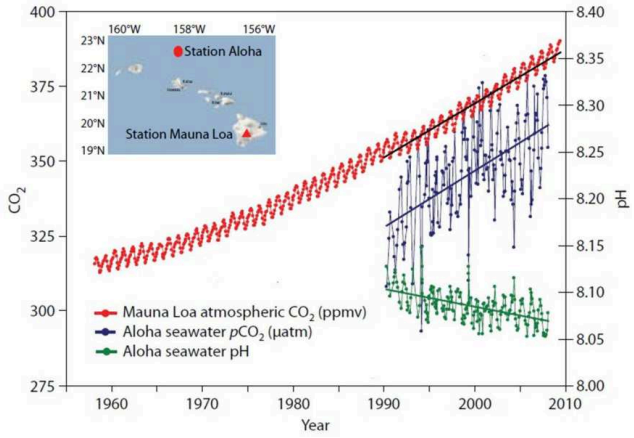

Fig. 1.1 Time series of atmospheric CO_2 concentration, surface ocean pH and surface ocean pCO_2 at Mauna Loha and Aloha stations, Hawaii (adapted from Feely et al. 2009).

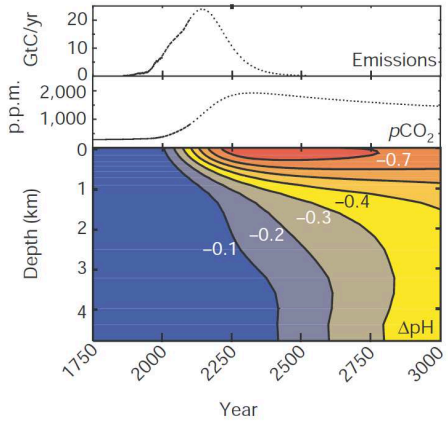

Fig. 1.2 CO_2 emissions scenario with predicted changes in atmospheric CO_2 and ocean pH (from Caldeira and Wickett 2003).

1.1.2 Carbon capture and sequestration

In an attempt to mitigate anthropogenic climate change, several geoengineering methods have been developed in order to reduce the CO_2 concentration in the atmosphere (reviewed by Shepherd 2009). A method considered to have a great potential is the so-called "carbon capture and sequestration" (CCS). According to this method, CO_2 should be captured and concentrated directly at the site of its production (mainly power plants) instead of releasing it to the atmosphere. Then, the CO_2 could be sequestered either in geological formations below the earth's surface, both onshore and offshore, or in the water column and/or on the seafloor of the world's oceans (Rubin et al. 2005). CCS might thus allow to reduce CO_2 emissions while still using fossil fuels (Schrag 2009).

While ocean storage of CO_2 was prohibited by the European Union due to the potential negative effects on the marine environment (EU 2009), the injection of CO_2 into underground geological formations such as oil and gas reservoirs, deep saline formations and unminable coal beds is already undertaken at a few sites worldwide, both onshore and offshore (Rubin et al. 2005). Carbon storage in offshore sediments

(Fig. 1.3) is considered to be a relatively safe method, especially at deep-sea sites, where CO_2 is denser than seawater due to the high pressure and low temperatures (Schrag et al. 2009). However, leakages from CO_2 storage sites cannot be ruled out over the long term (Hawkins 2004, Gerlagh and van der Zwaan 2012), and such leakages might result in severe reductions of local seawater pH (Widdicombe and Needham 2007).

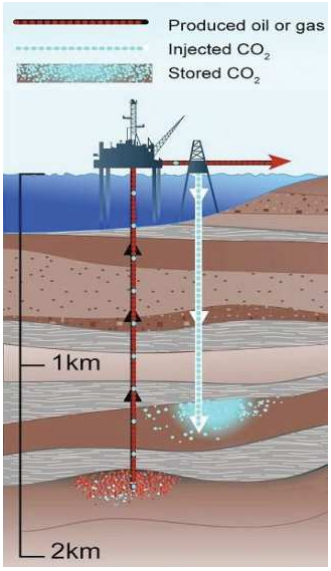

Fig. 1.3 Offshore sequestration of CO_2 in deep underground geological formations (adapted from Rubin et al. 2005).

1.2 Effects of elevated pCO_2 on marine life

Seawater acidification, whether caused by CO_2 uptake from the atmosphere or by leakages from sub-seabed storage sites, has been shown to severely affect a number of marine species, either directly or indirectly. At the beginning of OA research, most studies have focused on the responses of marine calcifiers such as coccolithophores, molluscs and corals. These organisms are believed to be the most threatened group in the face of OA, as the decrease in carbonate ion concentration (Box 1) can severely hamper their ability to build and maintain skeletal structures (e.g. Riebesell et al. 2000, Fabricius et al. 2011, Comeau et al. 2012). In recent years, however, evidence emerged that not only calcification, but also other processes in marine animals can be sensitive to an elevated seawater pCO_2, including e.g. negative effects on metabolic rates (e.g. Michaelidis et al. 2005, Rosa and Seibel 2008, Li and Gao 2012, Saba et al. 2012), reproduction (e.g. Havenhand et al. 2008, Kawaguchi et al. 2011, Zhang et al. 2011),

growth (e.g. Dupont and Thorndyke 2008, Kurihara et al. 2008, Fitzer et al. 2012), animal behavior (Ellis et al. 2009, Munday et al. 2009, 2010) and finally survival (e.g. Dupont et al. 2008, Kurihara et al. 2008, Zhang et al. 2011, Gonzales-Bernat et al. 2013). Thus, also the performance of non-calcifying species such as planktonic crustaceans can be directly influenced by OA (e.g. Kurihara et al. 2008, Kawaguchi et al. 2011, Zhang et al. 2011), and the sensitivity to elevated CO_2 concentrations can vary among closely related species (Zhang et al. 2011) and among different life stages within a species (e.g. Kurihara et al. 2004).

Indirect effects of OA, e.g. altered food regimes (Brussaard et al. 2013) or predator/prey interactions (Munday et al. 2010), are rarely studied, but likely pose another threat on marine animals, including those species that are able to cope with the direct effects of a reduced seawater pCO_2. Studies on the freshwater cladoceran *Daphnia pulicaria* and the calanoid copepod *Acartia tonsa* indicate that an altered chemical composition in the food algae, caused by OA, directly translates to the grazers and finally affects their growth and reproductive output (Urabe et al. 2003, Rossoll et al. 2012). In a mesocosm study, mesozooplankton organisms (*Cirripedia* larvae and *Calanus* spp.) showed reduced feeding rates at high compared to low CO_2 concentrations, possibly due to lower food availability or quality (de Kluijver et al. 2013). In the intertidal gastropod *Littorina littorea*, OA disrupts the capability to produce thicker shells in the presence of predator cues, increasing their vulnerability to predation. Subsequently, the snails exhibited an increased avoidance behaviour towards predator cues, which might affect their interactions with other organisms (Bibbi et al. 2007). In the coral reef fish *Amphiprion percula* and *Pomacentrus wardi*, in contrast, larvae reared at elevated CO_2 concentrations became attracted to predator cues, leading to a significantly increased mortality rate due to predation (Munday et al. 2010).

Ocean acidification does not occur in isolation, but comes in combination with other factors of climate change, e.g. ocean warming. It has been shown that increasing seawater temperatures might potentiate the effects of elevated CO_2 concentrations on marine animals. In the scleractinian coral *Stylophora pistillata*, for example, calcification was significantly affected when both, temperature and pCO_2 were elevated (Reynaud et al. 2003). In the Antarctic echinoid *Sterechinus neumayeri* and the coral *Acropora tenuis*, fertilization success was impaired under these conditions (Ericson et

al. 2012, Albright and Mason 2013). The barnacle *Elminius modestus* exhibited decreased growth rates when exposed to OA and ocean warming (Findlay et al. 2010). In the edible crab *Cancer pagurus*, the spider crab *Hyas araneus* and larvae of the sea urchin *Strongylocentrotus franciscanus*, elevated pCO_2 narrowed the thermal tolerance windows of the animals (Metzger et al. 2007, O'Donnell et al. 2009, Walther et al. 2009).

1.3 The Arctic *Calanus* species

Negative effects of OA on marine key species, whether direct or indirect, can have severe consequences for the ecosystem functioning. This study focuses on the effects of an elevated seawater pCO_2 on Arctic calanoid copepods, a group of non-calcifying planktonic crustaceans. Calanoid copepods are key components of the pelagic marine ecosystem and generally dominate the zooplankton community in terms of abundance and/or biomass, especially in high latitudes (e.g. Longhurst 1985, Fransz and Gonzalez 1997, Gislason and Astthorsson 1998, Auel and Hagen 2002).

Three *Calanus* spp. dominate the zooplankton biomass in Arctic waters: *Calanus finmarchicus* (Gunnerus, 1770), *C. glacialis* Jaschnov, 1955 (Fig. 1.4) and *C. hyperboreus* Krøyer, 1838. These species are important components of the lipid-based Arctic food web. As key herbivores, they provide the link between small primary producers and higher trophic levels such as carnivorous zooplankton (e.g. amphipods; Kraft et al. 2013), fish (e.g. Runge 1988, Beaugrand et al. 2003, Prokopchuk and

Fig. 1.4 The Arctic copepod *Calanus glacialis*.

Sentyabov 2006), whales (e.g. Wishner et al. 1988, Michaud and Taggart 2011) and seabirds (e.g. Węsławski et al. 1999, Wold et al. 2011).

C. finmarchicus is a boreal species with a widespread distribution. Its center of reproduction lies in the two cyclonic gyres in the Greenland Sea and in the Norwegian Sea. From here, part of the population is transported northward into the Arctic Ocean and the Barents Sea. Another part is carried southward, where copepods penetrate into the North Sea as well as into Davis Strait and Baffin Bay, from where they are transported further south along the North American shelf as far as to ~40° N (Marshall and Orr 1955a, Jaschnov 1970, Conover 1988). *C. glacialis* is of Arctic origin (Grainger 1961) and widespread in the Arctic Basin and the Arctic shelf seas (Jaschnov 1970). It also occurs in the Canadian Archipelago, Hudson Bay, Fox Basin, Baffin Bay and the Davis Strait, and is carried into the North Atlantic with the Labrador Current and the East Greenland Current, but it penetrates only marginally south of the polar front. In the Pacific region, it is carried into the Bering Sea, the Sea of Okhotsk and the northern part of the Japan Sea (Jaschnov 1970, Conover 1988). *C. hyperboreus* is also an Arctic species, and its distribution area includes the Arctic Ocean, the Canadian Archipelago, Hudson Bay and the Baffin Bay, from where it is transported as far south as the Gulf of Maine (Conover 1965, 1988, Dawson 1978, Hirche and Mumm 1992). It is also abundant in the Greenland Sea, from where it is transported southward into the Norwegian Sea (Conover 1988, Hirche 1991).

C. finmarchicus, *C. glacialis* and *C. hyperboreus* are well adapted to the environmental conditions prevailing in their respective habitats. To overcome food scarcity during the winter months, they overwinter in deep waters in a diapause stage. During this time, the copepods are torpid, their development is arrested, metabolic and digestive enzyme activities are low, and they rely on internal lipid reserves, which they accumulated during spring and summer (Conover and Corner 1968, Hallberg and Hirche 1980, Head and Conover 1983, Head and Harris 1985, Auel et al. 2003).

The smallest of the three species, *C. finmarchicus*, produces one to three generations per year, depending on the latitude (Marshall and Orr 1955a, Conover 1988). In fall, the lipid-rich copepodite stage V (CV) descents to deeper water layers to overwinter. Before the onset of the spring phytoplankton bloom, CV molt to adults and ascent to the

surface, where they start to reproduce. Gonad maturation and spawning are generally strongly correlated to the availability of food, however, egg production can already start at low rates before the phytoplankton bloom (Marshall and Orr 1955a, Hirche et al. 1997, Niehoff et al. 1999, Campbell et al. 2001). In contrast to *C. finmarchicus*, the medium-sized *C. glacialis* and the large *C. hyperboreus* from Arctic regions have multi-year life cycles (Tande et al. 1985, Conover 1988, Hirche 1997). *C. glacialis* seems to have a two-year life cycle in most of its distribution areas (Hirche 1998 and references therein). Overwintering CV molt to adults in late winter and ascent to the surface. Gonad maturation and egg production take place at a reduced rate during winter, depending on stored lipids and ice algae grazing. High reproductive rates, however, are only found during the phytoplankton bloom (Hirche and Kattner 1993, Niehoff et al. 2002). In the first year, the copepods develop to CIV, which is the first overwintering stage of *C. glacialis*. In the following year, CIV develop to CV, the second overwintering stage, and finally molt to adults. In *C. hyperboreus*, CV molt to adults in deep waters during winter. Egg production and spawning are completely fuelled by internal lipid reserves in this species and completed before the spring ascent (e.g. Hirche and Niehoff 1996). From CIII on, the copepods accumulate enough lipid reserves to successfully overwinter (Hirche 1998), and it takes 2-5 years for *C. hyperboreus* to complete its life cycle (Hirche 1998, Falk-Petersen et al. 1999).

Studies on the sensitivity of the three Arctic *Calanus* spp. to OA are still rare, and only the responses of active individuals have been investigated so far, indicating that hatching success, developmental time and survival of early life stages may be significantly affected at very high seawater pCO_2 > 3000 µatm (Mayor et al. 2007, Weydmann et al. 2012, Pedersen et al. 2013). Projected levels of OA, in contrast, do not appear to be harmful to these copepods (Marshall et al. 1935, Mayor et al. 2007, Weydmann et al. 2012, Lewis et al. 2013, Pedersen et al. 2013). Most of the studies, however, where short-term studies and did not exceed ten days. As the sensitivity to elevated pCO_2 can increase with increasing exposure time (Yamada and Ikeda 1999, Kurihara et al. 2008), longer-term studies are needed to assess the effects of OA on both active and diapausing *Calanus* spp. Large-scale studies on multi-species assemblages investigating indirect effects of OA on *Calanus* spp. are also rare (de Kluijver et al. 2013, Niehoff et al. 2013), but indicate that changes in the food availability and quality

may affect grazing in the copepods. No data are yet available on how Arctic *Calanus* spp. will cope with combined effects of elevated seawater pCO_2 and temperatures.

1.4 Aims and outline of the thesis

Due to anthropogenic influences, the Arctic regions are undergoing rapid changes, with possibly severe consequences for marine life. The calanoid copepods *Calanus finmarchicus, C. glacialis* and *C. hyperboreus* are key components in the Arctic marine ecosystem. By combining controlled long-term laboratory CO_2 incubation experiments and an ecosystem-scale mesocosm study, during which ecological as well as physiological responses of the copepods were monitored, this study aims to provide a comprehensive overview on the sensitivity of an important Arctic mesozooplankton group to ocean acidification.

The main objectives of this thesis were:

- to investigate whether and how different *Calanus* species and life stages respond to OA,
- to compare whether the sensitivity of active and diapausing life phases to climate change differs,
- to examine whether ocean warming and OA induce synergistic effects in *Calanus* spp.,
- to determine whether and how copepods respond to OA under near-natural conditions, when they can interact with a natural plankton community.

The study was carried out within the framework of BIOACID (Biological Impacts of Ocean Acidification), a joint research project of the German Federal Ministry of Education and Research (BMBF). Experiments and analyses, which are briefly summarized in section 2, were conducted at the facilities of the Alfred Wegener Institute, Helmholtz Center for Polar and Marine Research (AWI) in Bremerhaven, onboard RV *Polarstern* during cruises to the Fram Strait and at Espegrend, the Marine Biological Station of the University Bergen. The results of this study are presented in three publications (section 3). **Publication I** evaluates how body mass, respiration, gonad development and mortality of overwintering stages of *C. hyperboreus* and *C. glacialis* are affected during long-term exposure to elevated pCO_2, and if synergistic effects of elevated pCO_2 and temperatures occur. In **Publication II**, body mass and

grazing activity of active *C. glacialis* and *C. finmarchicus* were evaluated under OA scenarios, and **Publication III** describes results from a mesocosm study, tackling the effects of elevated seawater pCO_2 on the abundance and taxonomic composition of a near-natural zooplankton community. In section 4, the results of all experiments are summarized, and additional results on the acid-base status of overwintering *C. hyperboreus* as well as on respiration rates, body mass and egg production of *C. finmarchicus* from the mesocosm study are presented. In a joint discussion, the results gained during this study are combined with available literature data on other copepod species to assess the risk of two driving forces of climate change, namely ocean acidification and ocean warming, on this ecologically important zooplankton group.

2 MATERIALS AND METHODS

To study how Arctic *Calanus* spp. respond to OA, three laboratory and one mesocosm experiment were conducted. Detailed descriptions of the methods used are provided in the respective publication sections. In this chapter, these methods are briefly summarized, and additional methods are presented.

2.1 Copepod sampling

For the laboratory incubation experiments, *Calanus* spp. were sampled during three RV *Polarstern* expeditions to the Fram Strait in June/July 2010 (ARK-XXV/2), July/August 2011 (ARK-XXVI/2) and in June/July 2012 (ARK-XXVII/1 and 2). *C. finmarchicus* was sampled in the Norwegian Sea (**Publication II**), *C. glacialis* in the western part of the Fram Strait on the East Greenland continental shelf (**Publications I** and **II**), and *C. hyperboreus* in the area of the AWI HAUSGARTEN in the eastern Fram Strait (**Publication I**) (Fig. 2.1). Sampling was conducted with vertical bongo net hauls. During ARK-XXVI/2, additional individuals of *C. hyperboreus* were kindly provided from multi net hauls by A. Kraft (AWI). *Calanus* spp. then were maintained at ambient temperatures in natural seawater until transportation to the AWI laboratories in Bremerhaven or the start of onboard incubation experiments.

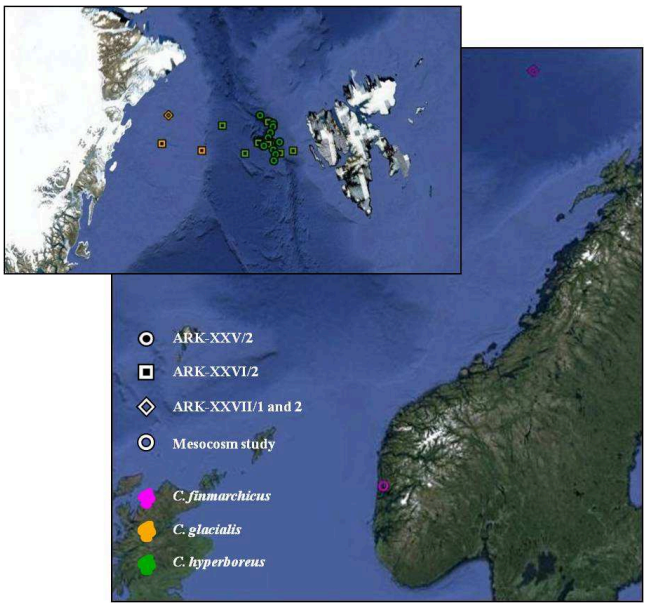

Fig. 2.1 Sampling area for the three *Calanus* spp. in the Norwegian Sea and in the Fram Strait (small map) during three RV *Polarstern* expeditions and a mesocosm study. Maps were obtained from Google Earth (Google Inc., 2013).

2.2 Incubation and subsampling

During the laboratory experiments, copepods were incubated in large containers (2 to 6 L) for 2 to 17 weeks in natural seawater which was adjusted to different CO_2 concentrations by aerating it with an air/CO_2 mix (Table 2.1). In addition, *C. hyperboreus* females were also incubated at different temperatures. Monocultures of the diatom *Thalassiosira weissflogii* were provided as food organisms, except for the CO_2/temperature experiment with diapausing *C. hyperboreus*, when copepods were not fed. In regular intervals, *Calanus* species and life stages were subsampled from the incubation containers, and body mass (dry weight and C and N content), respiration rates, mortality, gonad development, extracellular pH and grazing rates were determined in order to analyze their response to OA and ocean warming (**Publication II** and **III;** Table 2.1).

Table 2.1 Overview of the analyses and experiments conducted during laboratory CO_2 incubation experiments with Arctic *Calanus* spp.

					Analyses/Experiments						
Species/life stage	**Season**	**CO_2 (µatm)**	**Temp (°C)**	**Exp. time (d)**	**DW**	**CN**	**RespR**	**Mortality**	**GonD**	**pH_e**	**GrR**
C. hyperboreus CV	winter 2010	390/3000	0	63	x	x	x	x			
C. hyperboreus ♀	winter 2010/2011	390/3000	0/5/10	86-119	x	x	x	x	x	x	
C. glacialis CV	winter 2010	390/3000	0	62	x	x	x	x			
C. glacialis CV	summer 2011	390/1120/3000	0	16		x					x
C. finmarchicus CV	summer 2011	390/1120/3000	5	13		x					x

Temp = temperature; Exp. time = exposure time; DW = dry weight; CN = carbon and nitrogen content; RespR = respiration rate; GonD = gonad development; pH_e = extracellular pH; GrR = grazing rate; CV = copepodite stage V; ♀ = females

In May and June 2011, I participated in the SOPRAN (Surface Ocean Processes in the Anthropocene) mesocosm study at Espegrend, the Marine Biological Station of the University Bergen, Norway. During this study, the development of the mesozoo-plankton community at different pCO_2 levels was analyzed (**Publication III**), with an additional focus on the response of *C. finmarchicus* (**Publication II**, chapter 4).

Nine Kiel Off-Shore Mesocosms for future Ocean Simulation (KOSMOS) designed by the GEOMAR, Kiel, Germany (Fig. 2.2) were deployed in the Raunefjord (Fig. 2.1) for

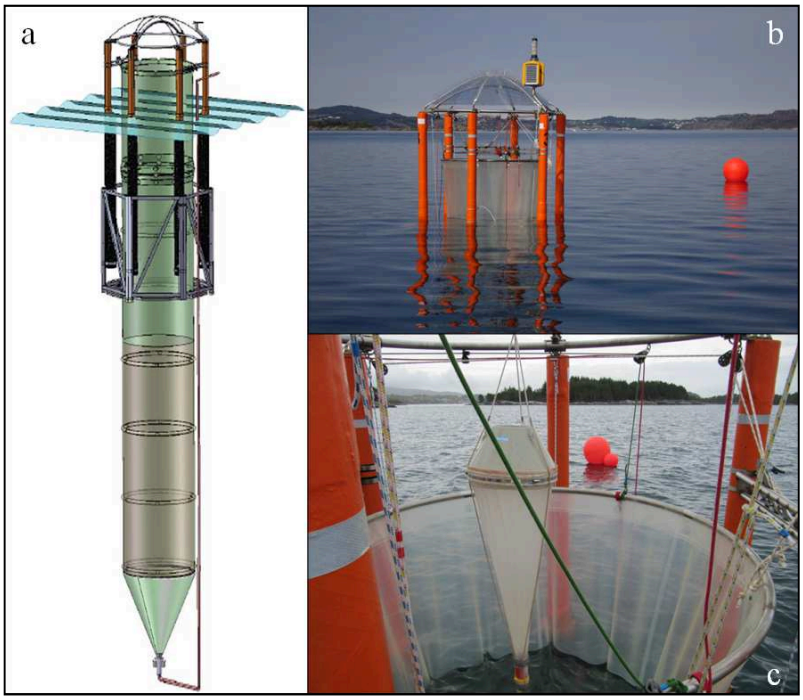

Fig. 2.2 KOSMOS mesocosms. (a) Sketch of a mesocosm, showing the floating frame with the unfolded mesocosm bag (not to scale) and the attached sediment trap (modified after Riebesell et al. 2013). (b) Visible part of a mesocosm floating in the Raunefjord (photo by N. Hildebrandt). (c) Sampling with an Apstein net (photo by D. Freese).

six weeks. Each mesocosm was 25 m in length and enclosed approx. 75 m^3 of fjord water containing natural plankton < 3 mm. Technical details on the mesocosms and their deployment are presented in Riebesell et al. (2013) and summarized in **Publication II** and **III**. Two mesocosms were kept at ambient conditions, which were ~280 µatm pCO_2 and pH 8.1 at the time of the experiment. In the remaining mesocosms, the pCO_2 was manipulated by adding CO_2 saturated fjord water in five steps over five consecutive days, starting on 8 May (t_0). Initial CO_2 concentrations after the manipulation was completed were 390, 560, 840, 1120, 1400, 2000 and 3000 µatm, resulting in pH values between 8.0 and 7.2 (K. G. Schulz (GEOMAR), unpubl. data). Over time, the pCO_2 decreased in the seven manipulated mesocosms due to outgassing and biological activities, however, there was still a gradient in pCO_2 present at the last sampling day (K. G. Schulz (GEOMAR), unpubl. data).

To study how the mesozooplankton community in general and *C. finmarchicus* in particular respond to the elevated seawater pCO_2, samples were regularly taken from the mesocosms and the fjord with an Apstein net (Fig. 2.2c, Table 2.2). Each week, one net

sample from each mesocosm and the fjord was preserved in 4 % formalin buffered with hexamethylenetetramine to analyze the mesozooplankton community. A second sample was filtrated on a combusted, pre-weighted GF/F filter to determine mesozooplankton biomass (**Publication III**). Two additional net hauls were conducted weekly to sort live *C. finmarchicus* CV and females to determine body mass, grazing rates (**Publication II**), respiration rates and egg production rates (EPR). In addition, subsamples of sedimented material collected daily from the mesocosms' sediment traps were provided by T. Boxhammer (GEOMAR) to analyze mesozooplankton abundance (**Publication III**).

Table 2.2 Number and purpose of net hauls conducted in each of the nine mesocosms and in the fjord during the SOPRAN mesocosm study.

Day	Number of net hauls	Analyses/Experiments		Remarks
		Zooplankton community	*Calanus finmarchicus*	
(5 May)	1		GrR, RespR	only fjord sampling
t_{-1}	3	A, BM	CN, EPR	before CO_2 manipulation (7 May)
t_5	3	A, BM	CN, EPR	after completion of CO_2 manipulation
t_7	1		GrR[1], RespR[2]	
t_{12}	3	A, BM	CN	
t_{14}	1		GrR[1], RespR[2], EPR	
t_{19}	3	A, BM	CN, EPR	
t_{21}	1		GrR[1], RespR[2]	
t_{25}	3	A, BM	CN, EPR	
t_{27}	1		GrR[1], RespR[2]	
t_{33}	3	A, BM	CN, EPR	

A = abundance; BM = biomass; CN = carbon and nitrogen content; EPR = egg production rates; GrR = grazing rates; RespR = respiration rates
[1]only for copepods from mesocosms initially adjusted to 390, 1400 and 3000 µatm CO_2
[2]only for copepods from mesocosms initially adjusted to 390 and 3000 µatm CO_2

2.3 Experiments and sample analyses

2.3.1 Respiration rates

To determine respiration rates (**Publication I,** chapter 4), 40 copepods from different CO_2 treatments were incubated for 3 to 22 hours in Winkler bottles containing filtrated seawater of the respective CO_2 concentration. At the start and at the end of the incubation, the oxygen saturation in the bottles was measured with oxygen microoptodes. To correct for bacterial oxygen consumption, additional blank bottles

without copepods were run. Respiration rates were calculated in terms of mL O_2 g dry weight^{-1} h^{-1}.

2.3.2 Grazing rates

To determine grazing rates (**Publication II**), 30 copepods from each CO_2 treatment were transferred to 1 L bottles containing CO_2 manipulated filtrated seawater inoculated with *T. weissflogii*. Together with blank bottles containing only seawater and algae, the bottles with the copepods were mounted to a slowly rotating plankton wheel for 17.5 to 25 hours. At the start and at the end of the experiment, subsamples of the incubation water were taken to analyze the chlorophyll *a* content, and grazing rates were calculated according to Frost (1972).

2.3.3 Extracellular pH

Extracellular pH (pH_e) in the hemolymph of *C. hyperboreus* females was measured in cold rooms adjusted to the respective incubation temperature. According to the method described in Schründer et al. (2013), five copepods from each CO_2/temperature treatment were placed on a petri dish under a stereo-microscope immediately after sampling them from the incubation containers. The copepods were carefully dried with tissue paper, and the hemolymph was extracted with pointed glass capillaries, which were inserted dorsally into the carapace. 500 nL of the hemolymph were mixed with 26 nL of the fluorescent pH indicator HPTS (95 µmol L^{-1}). The fluorescence of the hemolymph was then determined at 365 and 470 nm using a NanoDrop 3300 (Thermo Fisher Scientific, Waltham, MA, USA), and the ratio of the fluorescence values (365:470 nm) was calculated. pH_e was then calculated from a calibration curve with 50 mM Imidazole buffered seawater in the pH range from 5.0 to 8.5 (Schründer et al. 2013). Results are presented in chapter 4.

2.3.4 Female gonad development stage

The gonad development stage (GS) of female *C. hyperboreus* was examined in life individuals under a stereo microscope (**Publication I**). According to Hirche and Niehoff (1996) and Niehoff (1998), GS 1 describes immature females with empty diverticula. GS 2 and 3 characterize semi-mature females with increasing numbers of developing oocytes in the diverticula. Females in GS 4 carry mature oocytes and are ready to

spawn, whereas the diverticula of senescent females (S), which are at the end of the reproductive period, are thin bands, sometimes with single eggs left.

2.3.5 Egg production rates

EPR were measured in *C. finmarchicus* females during the SOPRAN mesocosm study. The results are presented in chapter 4. As females were rare in the fjord and in the mesocosms by the time of the experiment, all females were sorted from the net hauls. Beakers or cell well plates as often used in egg production measurements (e.g. Irigoien et al. 2000, Bonnet and Carlotti 2001, Niehoff 2003, Holste and Peck 2006) were not suitable in our experiments as they do not allow to keep the pCO_2 in the incubation water stable. Therefore, air-tight plastic culture flasks were used, which were cut into two pieces. The upper part (with lid) of one flask was covered with gauze (mesh size: 300 µm) and the upper part of a second flask was glued to that of the first flask. These egg production chambers (~85 ml) were filled with freshly sampled mesocosm water that was screened over a 55 µm mesh. Then, one to five females from the respective mesocosms were placed in the upper chamber and incubated for 18 to 21.5 hours. Eggs which were produced during the incubation sank through the net into the second chamber, where they were separated from the females and thus prevented from egg cannibalism (Runge and Roff 2000). At the end of the incubation, the lid of the lower chamber was opened and the water was filtered through a sieve (mesh size: 55 µm). Produced eggs were counted under a binocular, and EPR were calculated as eggs $female^{-1}$ day^{-1}.

2.3.6 Body mass

Body mass was regularly determined from copepods sampled from the incubation containers and mesocosms as well as from the grazing, respiration and egg production experiments (**Publication I** and **II**, chapter 4). The prosome length was measured under a stereo microscope. Then, the copepods were rinsed in distilled water and deep-frozen at -20 °C. To measure dry mass, they were dried and weighted on an ultra-microbalance. C and N content were then determined with an elemental analyzer.

2.3.7 Mortality

Copepods that had died in the incubation containers became opaque and were thus easily distinguishable from live ones. Dead specimen were removed regularly and counted in order to calculate mortality (**Publication I**).

2.3.8 Zooplankton abundance in the mesocosms

To analyze the mesozooplankton abundance and community composition in the water column and sediment traps of the mesocosms (**Publication III**), organisms in the samples were determined to the lowest taxonomical level, if possible to species and developmental stage (copepods). Sediment trap samples were analyzed completely, and abundances were calculated as total numbers of organisms collected 2 $days^{-1}$. The water samples were splitted with a plankton splitter into subsamples of 1/8, 1/16 or 1/32, and zooplankton organisms were determined in at least one subsample. Abundances were calculated as individuals m^{-3}. Mesozooplankton biomass in the mesocosms was determined by drying the filters with the zooplankton samples and weighting them to calculate dry weight m^{-3}.

2.4 Statistics

Data were analyzed using the software SigmaStat 3.5 (Systat Software, Inc.) and R (version 3.02; R Core Team 2013). Spearman Rank tests were performed to test whether the body mass of the three *Calanus* spp. changed over the course of the different experiments (**Publication I** and **II**, chapter 4), whether the abundance of *C. finmarchicus* and other zooplankton groups in the mesocosms was affected by time and/or CO_2 (**Publication III**, chapter 4), and whether the grazing rates of the copepods were significantly correlated to the initial chl *a* concentration at the start of the grazing experiments (**Publication II**). To test for differences in body mass, respiration rate, grazing rate or EPR among copepods from different CO_2 treatments (**Publication I** and **II**, chapter 4), data from each sampling/experimental day were compared using either t-tests (normally distributed data, two CO_2 levels), Mann-Whitney Rank Sum tests (non-normally distributed data, two CO_2 levels), one-way ANOVA followed by post-hoc Holm-Sidak tests (normally distributed data, three CO_2 levels) or Kruskal-Wallis tests followed by post-hoc Tukey tests (non-normally distributed data, three CO_2 levels). To test for combined effects of CO_2 and temperature on body mass and pH_e of *C. hyperboreus* females (**Publication I**, chapter 4), two-way ANOVA followed by post-

hoc Holm-Sidak tests were performed. When the data were not normally distributed, they were $\log_{10}$ transformed before the analysis. Z-tests were used to compare the proportion of dead copepods as well as the proportion of the different female gonad developmental stages between CO_2 and CO_2/temperature treatments (**Publication I**). To test for changes in the zooplankton community in the water column and the sediment traps of the mesocosms, linear mixed effect models were fitted to the Shannon-index H, which is a measure for diversity (**Publication III**). Data were considered significantly different at a $p < 0.05$. Results are presented as mean ± standard deviation.

3 PUBLICATIONS

PUBLICATION I

Hildebrandt, N., Niehoff, B. and Sartoris, F. J. (Marine Pollution Bulletin, in press)

Long-term effects of elevated CO_2 and temperature on the Arctic calanoid copepods *Calanus glacialis* and *C. hyperboreus*

The design of the study was developed by all authors. I sampled the copepods onboard RV *Polarstern*, carried out the experiments, analyzed the samples and wrote the manuscript, which was revised in cooperation with B. Niehoff and F. J. Sartoris.

PUBLICATION II

Hildebrandt, N., Sartoris, F. J., Schulz, K. G., Riebesell, U. and Niehoff, B. (submitted to Journal of Plankton Research)

Effects of ocean acidification on grazing of *Calanus finmarchicus* and *C. glacialis* (Copepoda: Calanoida)

The study design was developed by B. Niehoff, F. J. Sartoris and myself. During the mesocosm study, I conducted zooplankton sampling with support of U. Riebesell and K. G. Schulz and performed the grazing experiments. K. G. Schulz contributed mesocosm data. Copepod sampling and experiments on-board RV *Polarstern* were conducted by myself and B. Niehoff. I analyzed all data and wrote the manuscript, which was revised together with all co-authors.

PUBLICATION III

Hildebrandt, N., Sartoris, F. J., Czerny, J., Büdenbender, J., Boxhammer, T., Schulz, K. G. and Niehoff, B.

Ocean acidification effects on a boreal mesozooplankton community - a mesocosm study

The design of the study was developed by B. Niehoff, F. J. Sartoris and myself. I conducted the zooplankton sampling and analyses. T. Boxhammer provided sediment trap samples, J. Czerny and K. G. Schulz contributed mesocosm data. I wrote the manuscript, which was revised together with F. J. Sartoris and B. Niehoff. J. Büdenbender, J. Czerny, T. Boxhammer and K. G. Schulz supported field work and commented on the manuscript.

PUBLICATION I

Long-term effects of elevated CO_2 and temperature on the Arctic calanoid copepods *Calanus glacialis* and *C. hyperboreus*

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In Press

ABSTRACT

The sensitivity of copepods to ocean acidification (OA) and warming may increase with time, however, studies >10 days and on synergistic effects are rare. We therefore incubated late copepodites and females of two dominant Arctic species, *Calanus glacialis* and *C. hyperboreus,* at 0 °C at 390 and 3000 µatm pCO_2 for several months in fall/winter 2010. Respiration rates, body mass and mortality in both species and life stages did not change with pCO_2. To detect synergistic effects, in 2011 *C. hyperboreus* females were kept at different pCO_2 and temperatures (0, 5, 10 °C). Incubation at 10 °C induced sublethal stress, which might have overruled effects of pCO_2. At 5 °C and 3000 µatm, body carbon was significantly lowest indicating a synergistic effect. The copepods, thus, can tolerate pCO_2 predicted for a future ocean, but in combination with increasing temperatures they could be sensitive to OA.

KEYWORDS: Ocean acidification • Ocean warming • Respiration • Body mass • Gonad development • Mortality

1 INTRODUCTION

Ocean acidification (OA), i.e. the uptake of atmospheric CO_2 by the oceans and the subsequent decline in pH, and ocean warming may severely affect the performance of marine organisms and their trophic interactions (e.g. reviews by Fabry et al. 2008, Richardson 2008). OA can thus be an important driver influencing marine ecosystem processes to date and in future (e.g. Raven et al. 2005, Solomon et al. 2007). With elevated seawater pCO_2, the carbonate concentration decreases (e.g. Raven et al. 2005), and thus, most studies at the beginning of OA research have focused on marine calcifiers (e.g. Riebesell et al. 2000, Orr et al. 2005). Recent studies have, however, shown that not only calcification processes but also reproduction, growth and behavior can be affected by elevated seawater pCO_2 (e.g. Munday et al. 2010, Kawaguchi et al. 2011, Fitzer et al. 2012, see review in Wittmann and Pörtner 2013, their Fig. 1). As a consequence, increasing attention has recently been paid to the response of soft-bodied organisms to OA.

Among the non-calcifying zooplankton organisms, calanoid copepods play a key role in pelagic marine ecosystems as they account for up to 80 % of the zooplankton biomass (Longhurst 1985), link primary production to higher trophic levels (e.g. Runge 1988, Węsławski et al. 1999) and contribute to the carbon transport from the surface to the deep sea (e.g. Schnack-Schiel and Isla 2005). Short-term laboratory studies ($\leq$ 10 days) which investigated the ecological effects of OA on calanoid copepods indicated that mortality, development, egg production rates and hatching success may be impaired by CO_2 partial pressures (pCO_2) $\geq$ 2000 µatm, whereas pCO_2 levels predicted for the end of the century seem to have no impact on most of the copepod species yet studied (Kurihara et al. 2004, Watanabe et al. 2006, Mayor et al. 2007, Kurihara and Ishimatsu 2008, Zhang et al. 2011, Mayor et al. 2012, Vehmaa et al. 2012, Weydmann et al. 2012, McConville et al. 2013). Only in *Centropages tenuiremis*, a pCO_2 of 1000 µatm affected respiration and grazing rates during a 90 h incubation experiment (Li and Gao 2012).

The sensitivity of marine animals to high pCO_2 and low pH can increase with exposure time (Yamada and Ikeda 1999, Kurihara et al. 2008). Long-term studies are thus crucial to evaluate the impact of OA on copepods, however, to date such studies are rare. In a 30-day mesocosm experiment in an Arctic fjord, the impact of OA on a plankton

community was studied under near-natural conditions (Riebesell et al., 2013). During this study abundance and developmental stage composition of *Calanus* spp., *Acartia longiremis*, *Oithona similis* and *Microsetella norvegica* were not affected by increasing pCO_2 (185 to 1420 µatm) (Niehoff et al. 2013). However, the grazing rates of *Calanus* spp. decreased with increasing pCO_2 (de Kluijver et al. 2013), but it cannot be distinguished whether this was due to indirect (via trophic interactions) or direct effects of OA. In laboratory experiments, CO_2 incubations for 20 days and longer have only been conducted with three copepod species. In *Acartia tsuensis*, the effects of OA were studied over two generations (20 days), revealing that a pCO_2 of 2380 µatm (pH = 7.32) had no influence on egg production and hatching rate (Kurihara and Ishimatsu 2008). In contrast, growth and reproductive rates of the harpacticoid copepod *Tisbe battagliai*, incubated for four generations (approx. 56 days), decreased at a $pCO_2 \geq 394$ µatm and a corresponding pH $\leq$ 7.82 (Fitzer et al. 2012). Just recently, Pedersen et al. (2013) published a study on boreal *Calanus finmarchicus,* raised from eggs to adults at very high pCO_2, showing that the generation time increased at ≥7300 µatm.

In Arctic waters, *Calanus glacialis* Jaschnov, 1955 and *C. hyperboreus* Krøyer, 1838 dominate the mesozooplankton biomass and are major components of the food web. *C. glacialis* is a polar species (Grainger 1961) and inhabits the Arctic shelf regions, from where it is transported into the Fram Strait with the East Greenland Current (Jaschnov 1970, Conover 1988). The main distribution areas of *C. hyperboreus* are the Greenland Sea, the Canadian Archipelago and the Baffin Bay (Conover 1988, Hirche 1991). It also occurs in the Arctic Ocean (Dawson 1978, Hirche and Mumm 1992) and is advected southwards into the Norwegian Sea and the Gulf of Maine (Conover 1965, 1988). Both, *C. glacialis* and *C. hyperboreus* have multi-year life cycles in Arctic waters (e.g. Tande et al. 1985, Conover 1988, Hirche 1997). From copepodite stage III (*C. hyperboreus*) and stage IV (*C. glacialis*) on (Hirche 1998), the species spend the period of scarce food in winter in diapause in deep waters (*C. glacialis*: 200-300 m (Falk Petersen et al. 2007); *C. hyperboreus*: 500-3000 m (Hirche 1991)). During this time the copepods are torpid, their development is arrested, metabolic and digestive enzyme activities are low, and they rely on internal lipid reserves only (Conover and Corner 1968, Hallberg and Hirche 1980, Head and Conover 1983, Head and Harris 1985, Auel et al. 2003). If the copepods experienced elevated temperatures during this resting stage due to ocean warming, the lipid stores may faster be depleted and, thus, the maximum duration of the

diapause may be shortened as models suggest for *C. finmarchicus* (Saumweber and Durbin 2006, Pierson et al. 2013). Also, exposure to elevated CO_2 concentrations can affect the energy budget (Li and Gao 2012). As diapausing copepods cannot compensate for energetic losses, they might be especially vulnerable to climate change.

In both *C. glacialis* and *C. hyperboreus*, reproduction and survival have been studied during short-term exposure to high pCO_2 (Weydmann et al. 2012, Lewis et al. 2013) while there is no information on effects during long-term exposure. Also, no data are available on the response of diapausing individuals and, in addition, there are no studies on synergistic effects of ocean warming and OA on these two species. The aims of this study were therefore (1) to compare the long-term responses of two Arctic *Calanus* spp. to elevated seawater pCO_2 as well as temperatures during fall/winter and (2) to detect possible synergistic effects of OA and ocean warming. To get a comprehensive view on different ecological and physiological parameters, we measured mortality, respiration rates, body mass in terms of carbon and nitrogen content and, in females, gonad development during incubation experiments with copepodite stage V (CV) of *C. glacialis* and *C. hyperboreus* and with female *C. hyperboreus* over two to four months at pCO_2 of 390 and 3000 µatm and 0 °C. To study synergistic effects of elevated pCO_2 and temperatures, in an additional experiment *C. hyperboreus* females were incubated at different temperatures (0, 5 and 10 °C).

2 METHODS

2.1 Field work

The copepods were collected with vertical bongo and multi net hauls during two expeditions with RV *Polarstern* to the Fram Strait, ARK-XXV/2 in June/July 2010 and ARK-XXVI/2 in July/August 2011 (see Table 1 for details). Immediately after capture, the samples were diluted in surface seawater.

In 2010, *Calanus* spp. were picked by eye from the samples with glass pipettes and maintained in filtered seawater at 0 °C for up to four weeks. Every two to three days, the copepods were fed with natural phytoplankton that was sampled from the chlorophyll *a* maximum layer with a rosette. For transport in a cooling box by airplane,

Table 1 List of stations during two RV *Polarstern* cruises for sampling *Calanus glacialis* and *C. hyperboreus*.

Date	Station	Position		Net	Mesh size (µm)	Sampling depth (m)	Temp (°C)
		Lat (degmin)	Lon (degmin)				
03.07.2010	PS 76/109	78° 50.01' N	8° 0.35' E	Bongo	500	300	3.2 – 6.1
05.07.2010	PS 76/130	79° 5.10' N	4° 18.24' E	Bongo	500	300	N/A
05.07.2010	PS 76/130	79° 5.32' N	4° 17.83' E	Bongo	500	1000	N/A
10.07.2010	PS 76/159	78° 48.34' N	6° 0.31' E	Bongo	500	1500	-0.7 – 6.6
11.07.2010	PS 76/170	79° 8.57' N	2° 45.63' E	Bongo	500	300	0.4 – 5.3
14.07.2010	PS 76/180	79° 44.09' N	4° 29.29' E	Bongo	500	300	1.5 – 5.9
17.07.2010	PS 76/197	78° 49.95' N	0° 42.00' E	Bongo	500	300	3.2 – 6.1
19.07.2010	PS 76/208	79° 37.37' N	3° 0.23' W	Bongo	500	300	*-1.8 – -1.1
23.07.2010	PS 76/227	78° 50.24' N	5° 50.24' W	Bongo	500	314	-1.8 – 1.9
23.07.2010	PS 76/239	78° 50.27' N	11° 58.27' W	Bongo	500	150	-1.7 – -0.0
14.07.2011	PS 78/140	79° 7.85' N	6° 4.56' E	Bongo	200/300	500	1.9 – 5.6
16.07.2011	PS 78/142	79° 7.96' N	4° 53.03' E	Bongo	200/300	1000	-1.5 – 4.7
17.07.2011	PS 78/145	79° 8.58' N	2° 45.30' E	Bongo	200/300	100	-1.5 – 4.4
20.07.2011	PS 78/154	79° 6.64' N	4° 37.22' E	Bongo	300/1000	1000	-1.5 – 3.8
21.07.2011	PS 78/158	79° 2.76' N	3° 37.28' E	Bongo	300/1000	250	-1.4 – 3.7
21.07.2011	PS 78/159	79° 3.49' N	3° 28.88' E	Bongo	300/1000	250	-1.4 – 4.8
23.07.2011	PS 78/162	79° 45.48' N	4° 26.49' E	MN	1000	2000	-0.8 – 2.8
24.07.2011	PS 78/165	79° 16.84' N	4° 19.84' E	Bongo	300/1000	200	-0.6 – 4.0
25.07.2011	PS 78/166	79° 25.63' N	4° 44.38' E	MN	1000	2000	-0.8 – 4.5
25.07.2011	PS 78/166	79° 25.62' N	4° 44.28' E	Bongo	300/1000	200	-0.6 – 4.5
26.07.2011	PS 78/168	79° 56.48' N	3° 12.36' E	Bongo	300/1000	200	2.4 – 3.7
26.07.2011	PS 78/170	79° 40.96' N	5° 17.36' E	Bongo	300/1000	200	**-0.3 – 3.0
27.07.2011	PS 78/171	79° 35.72' N	5° 13.35' E	MN	1000	2000	-1.6 – 4.8
27.07.2011	PS 78/171	79° 35.71' N	5° 13.33' E	Bongo	300/1000	200	-1.6 – 4.8
28.07.2011	PS 78/174	78° 35.98' N	5° 0.01' E	Bongo	300/1000	200	2.7 – 7.1
29.07.2011	PS 78/176	78° 54.89' N	5° 1.16' E	Bongo	300/1000	200	2.4 – 5.3
29.07.2011	PS 78/181	78° 46.78' N	5° 19.48' E	Bongo	300/1000	200	3.5 – 7.7

Lat: Latitude; Lon: Longitude; Temp: Temperature; Bongo: Bongo Net (Hydro-Bios, Kiel, Germany); MN: MultiNet Type Maxi (Hydro-Bios, Kiel, Germany); N/A: data not available.
Temperature values indicate the temperature range of the sampled depth layer (CTD measurements: Beszczynska-Möller & Wisotzki (2010), Klages & Rohardt (2011)).
* Measurements only down to 106 m
** Measurements only down to 101 m

the copepods were transferred to 1 and 2 liter plastic bottles. At the Alfred Wegener Institute, *C. glacialis* and *C. hyperboreus* were sorted under a binocular and separated according to sex and developmental stage. The copepodite stage V (CV) was abundant in both species as were female *C. hyperboreus.* Therefore, these three groups were used in our first experiment. In 2011, only *C. hyperboreus* females were sorted onboard and

kept for up to three weeks at 0 °C until transport to the Alfred Wegener Institute. No food was provided as *the females* in the previous experiment did not feed and entered diapause.

2.2 Preparation of CO_2 manipulated seawater

The copepods were incubated in natural seawater with 390 (control) and 3000 µatm CO_2 (high pCO_2). To adjust the seawater to the respective pCO_2, it was aerated for about 24 h with an air/CO_2 mix, which was produced by a gas-mixing pump (HTK, Hamburg, Germany; accuracy: ± 1-5 %). Temperature and salinity were determined with a conductivity meter (WTW Cond340i). The pH was measured with a pH electrode (Mettler Toledo InLab Routine Pt1000, connected to a pH meter (WTW pH 3310)) that was calibrated with NIST buffers (pH 6.865 and 9.180) at the respective temperatures. In addition, the pH of a Dickson buffer was measured to convert the seawater pH to free scale (pH_F). The amount of dissolved inorganic carbon (DIC) in the seawater was determined with a Technicon Analyzer TrAAcs 800 (Seal Analytical GmbH, Norderstedt, Germany). The pCO_2 of the seawater was then calculated from pH_F and DIC using the program CO2SYS (Lewis and Wallace 1998).

2.3 Incubation experiments

In the first experiment from August through November 2010, 149 CV of *C. glacialis*, 95 CV of *C. hyperboreus* and 124 females of *C. hyperboreus* were incubated in the dark at 0 °C and CO_2 concentrations of 390 µatm and 3000 µatm CO_2, respectively (for water parameters see Table 2). The copepods in each group were distributed among three incubation containers. The CV of both species were kept in 2 L plastic containers, while *C. hyperboreus* females were kept in 6 L plastic tons due to their bigger size. *C. glacialis* CV were incubated for 62 days, *C. hyperboreus* CV for 63 days and *C. hyperboreus* females for 86 days. Differences in the incubation time are due to differing copepod numbers and mortality rates. Twice a week about 80 % of the water was gently removed from the incubation containers with a tube covered by 300 µm mesh and replaced by fresh CO_2 manipulated water (1) to compensate for losses of CO_2 due to out-gassing and algal growth and (2) to ensure O_2 saturation of > 80%. To account for changes in pCO_2, the pH and DIC of the water in the incubation containers were measured before and after the water exchange. The pH changed only slightly between

Table 2 Water parameters measured during the incubations.

Species	Treatment	Temp (°C)	Salinity (psu)	pH (free scale)	DIC (µmol l^{-1})	pCO_2 (µatm)
C. glacialis CV	Control	0.6 ± 0.7	32.3 ± 0.6	8.164 ± 0.039	2279 ± 51	334 ± 19
C. glacialis CV	High CO_2	0.6 ± 0.7	32.1 ± 0.4	7.243 ± 0.056	2495 ± 140	2981 ± 391
C. hyperboreus CV	Control	0.6 ± 0.7	32.3 ± 0.6	8.152 ± 0.051	2282 ± 48	346 ± 40
C. hyperboreus CV	High CO_2	0.6 ± 0.7	32.1 ± 0.4	7.223 ± 0.045	2482 ± 40	3099 ± 331
C. hyperboreus ♀	Control	0.5 ± 0.6	32.2 ± 0.5	8.142 ± 0.039	2273 ± 56	349 ± 25
C. hyperboreus ♀	High CO_2	0.6 ± 0.6	32.2 ± 0.4	7.232 ± 0.053	2480 ± 39	3089 ± 394
C. hyperboreus ♀	Control	0.2 ± 0.4	31.5 ± 0.9	8.070 ± 0.066	2299 ± 56	440 ± 74
C. hyperboreus ♀	High CO_2	0.2 ± 0.5	31.7 ± 0.9	7.265 ± 0.078	2466 ± 40	2892 ± 470
C. hyperboreus ♀	Control	4.7 ± 0.7	32.1 ± 1.4	8.105 ± 0.090	2319 ± 69	413 ± 55
C. hyperboreus ♀	High CO_2	4.7 ± 0.7	32.1 ± 1.7	7.304 ± 0.061	2499 ± 126	2817 ± 392
C. hyperboreus ♀	Control	9.5 ± 0.5	31.5 ± 1.0	8.103 ± 0.134	2280 ± 53	451 ± 58
C. hyperboreus ♀	High CO_2	9.4 ± 0.5	31.6 ± 1.0	7.337 ± 0.107	2457 ± 118	2804 ± 603

Values are presented as mean ± SD. Temp: temperature.

two water exchanges. At control pCO_2, the pH decreased on average by 0.006 ± 0.040 units, while it increased by 0.037 ± 0.047 units at high pCO_2. When fresh water was added, the copepods were also fed with the diatom *Thalassiosira weissflogii* at concentrations of 4000 to 8000 cells mL^{-1}. The algae were grown in f/2 medium (Guillard 1975) at 14 °C and constant light. Before fed to the copepods, the algal suspension was cooled to 0 °C and concentrated by inverse filtration to minimize dilution of the CO_2 enriched water. On days without feeding, the incubation containers were gently turned upside down to re-suspend the algae.

The second experiment from August through December 2011 was conducted to detect combined effects of elevated pCO_2 and increasing water temperatures. Female *C. hyperboreus* were incubated in the dark in 6 L gas-tight glass bottles (Schott) at 390 and 3000 µatm CO_2 and 0, 5 and 10 °C, respectively (Table 2). 0 °C is typical of the natural environment of *C. hyperboreus* during winter, whereas 10 °C slightly exceeds the upper temperature in its southernmost distribution area, the Gulf of Maine (2 - 8 °C; Conover and Corner 1968). The females were incubated in four groups of forty individuals at each CO_2/temperature combination. The incubation bottles with the copepods were warmed at a rate of 1 °C per 3 hours in a climatic test cabinet (Rumed, Rubarth Apparate GmbH, Laatzen, Germany). After the target temperatures were reached, the copepods were allowed to acclimate to the conditions for about 30 h. Then the water in the incubation bottles was removed and replaced with CO_2 manipulated water. The females were kept under experimental conditions for 119 days. During the first four weeks, the incubation water was changed every other day. Due to decreasing copepod

numbers, the frequency of water exchanges was then gradually reduced to finally weekly changes for the last eight weeks. The pH was almost stable between two water exchanges (-0.056 ± 0.109 units at control pCO_2; -0.018 ± 0.093 units at high pCO_2).

2.4 Sampling during the incubations

During all experiments, copepods were repeatedly sampled from the incubation bottles with glass tubes. To determine body mass, i.e. dry weight, carbon (C) content and nitrogen (N) content, 12 copepods from every treatment were measured (prosome length, ± 0.05 mm) under a stereo-microscope, shortly rinsed in distilled water, placed in tin cartridges and deep-frozen. For the analyses, the samples were dried at 60 °C for 2 days and weighted on an ultra-microbalance (Mettler Toledo, Gießen, Germany). C and N content were then measured with an elemental analyzer (Euro EA, HEKAtech GmbH, Wegberg, Germany) using acetanilide as a standard. In *C. hyperboreus* females, gonad development stages (GS) were determined after Hirche and Niehoff (1996) and Niehoff (1998): GS 1 describes immature females with empty diverticula. GS 2 and 3 characterize semi-mature females with increasing numbers of developing oocytes in the diverticula. Females in GS 4 carry mature oocytes and are ready to spawn, whereas the diverticula of senescent females (S), which are at the end of the reproductive period, are thin bands, sometimes with single eggs left. For respiration rate measurements, the copepods were immediately transferred into Winkler bottles (see below). To determine mortality in terms of percentage of copepods which had died during the incubation, dead animals were counted and removed from the bottles during every water exchange.

2.5 Respiration measurements

For respiration measurements, a sealed chamber method was used (Ikeda et al. 2000). 40 copepods from each pCO_2 and pCO_2/temperature treatment were placed in groups of ten in four 60 ml Winkler bottles filled with filtered seawater of the respective pCO_2 and temperature. An additional Winkler bottle containing only filtered seawater was run as a control for each treatment to correct for bacterial oxygen consumption. The Winkler bottles were placed in open-bath circulators with temperature control. The oxygen saturation in the water was measured at the beginning and at the end of an incubation with oxygen microoptodes (PreSens GmbH, Regensburg, Germany; accuracy: ± 0.4 % O_2 at 20.9 % O_2) connected to a 4-channel microsensor oxygen meter (PreSens GmbH).

The microoptodes were calibrated using seawater as a 100% O_2 reference and a saturated Na_2SO_3 solution as an oxygen-free standard. Depending on the copepod size and temperature, incubation times differed between the two species and the two developmental stages, ranging from three (*C. hyperboreus* females incubated at 10 °C) to 21 h (*C. glacialis* CV at 0 °C) to ensure that the O_2 saturation in the water did not fall short of 80 %. Mortality during the respiration measurements was very low. Of all copepods used, only one individual died throughout the measurement, and the respective Winkler bottle was excluded from the calculation of O_2 consumption.

As the O_2 consumption is related to size, specific respiration rates were calculated in terms of mL O_2 g dry weight^{-1} h^{-1}. In 2010, the copepods were not analyzed for body mass after respiration measurements but gently transferred back into the incubation containers. For calculations of mass-specific oxygen consumption we used the mean dry weight from the sampling that was closest to the respiration experiment. In 2011 total copepod numbers were higher than in 2010 and female mortality was low. Thus, after the respiration experiment twelve of the 40 copepods from each group were analyzed for length and gonad development stage and then deep-frozen for later analysis of dry weight and carbon and nitrogen content.

2.6 Statistics

Data were analyzed using SigmaStat 3.5 (Systat Software, Inc.). To test whether dry weight, C and N content and C:N ratio changed over the course of the two experiments, Spearman rank tests (SR) were performed. A t-test (for normally distributed data) or a Mann-Whitney rank sum test (for non-normally distributed data) was used to identify differences in respiration rates and body mass between copepods from control and high pCO_2 treatments. To test for combined effects of pCO_2 and species/developmental stage and of pCO_2 and temperature on body mass and respiration, two-way ANOVA, followed by post-hoc Holm-Sidak tests, were performed. When the data were not normally distributed, they were $\log_{10}$ transformed before the analysis. To compare the proportion of dead copepods as well as the proportion of the five gonad developmental stages between CO_2 and temperature treatments, z-tests were performed. Differences were considered significant at a $p < 0.05$. Results are given as mean ± standard deviation unless otherwise noted.

3 RESULTS

3.1 Effects of elevated pCO_2 on *Calanus glacialis* and *C. hyperboreus*

In 2010, we studied the response of the two copepod species *C. glacialis* and *C. hyperboreus* and two developmental stages (CV and females) of *C. hyperboreus*. This experiment revealed significant differences between the species and developmental stages in body mass and respiration as expected but there was no negative influence of pCO_2 in any of the three groups (two-way ANOVA, Table 3).

Table 3 Two-way ANOVA results for the effect of copepod species/developmental stage and seawater pCO_2 on *Calanus glacialis* CV, *C. hyperboreus* CV and *C. hyperboreus* females.

		DF	SS	MS	F	P
Dry weight	**species/stage**	**2**	**19.702**	**9.851**	**357.861**	**<0.001**
	CO_2	1	0.004	0.004	0.162	0.688
	species/stage * CO_2	2	0.124	0.062	2.260	0.107
C content	**species/stage**	**2**	**12.820**	**6.410**	**189.184**	**<0.001**
	CO_2	1	0.013	0.013	0.395	0.531
	species/stage * CO_2	2	0.010	0.005	0.141	0.868
N content	**species/stage**	**2**	**15.118**	**7.559**	**374.918**	**<0.001**
	CO_2	1	0.009	0.009	0.436	0.510
	species/stage * CO_2	2	0.001	0.001	0.030	0.970
C:N	species/stage	2	19.520	9.760	6.604	0.002
	CO_2	1	0.028	0.028	0.019	0.891
	species/stage * CO_2	2	1.778	0.889	0.601	0.549
Respiration	species/stage	2	0.748	0.374	36.486	<0.001
	CO_2	1	0.005	0.005	0.496	0.483
	species/stage * CO_2	2	0.012	0.006	0.594	0.554

Significant effects are marked in **bold**.

3.1.1 Body mass development

The body mass of *Calanus glacialis* CV and *C. hyperboreus* CV and females did not differ between copepods kept at control and high pCO_2 but changed with time (Fig. 1). At the start of the experiment, *C. glacialis* CV (prosome length 3.3 ± 0.3 mm) had a dry weight of 0.52 ± 0.24 mg, a C content of 329 ± 126 µg and an N content of 42 ± 12 µg. Over the course of the experiment, body mass increased in both treatment groups, however, significant differences were found only in the dry weight of copepods kept at high pCO_2 (SR, $p < 0.001$). The C:N ratio (7.2 ± 1.1) did not change significantly. *C. hyperboreus* CV (prosome length 5.1 ± 0.4 mm) had a dry weight of 2.46 ± 0.79 mg, a C content of 1511 ± 531 µg and an N content of 165 ± 54 µg at the start of the incubation. In *C. hyperboreus* females (prosome length 6.6 ± 0.3 mm), dry weight and C and N content were 4.23 ± 1.68 mg, 2537 ± 1081 µg and 300 ± 99 µg, respectively.

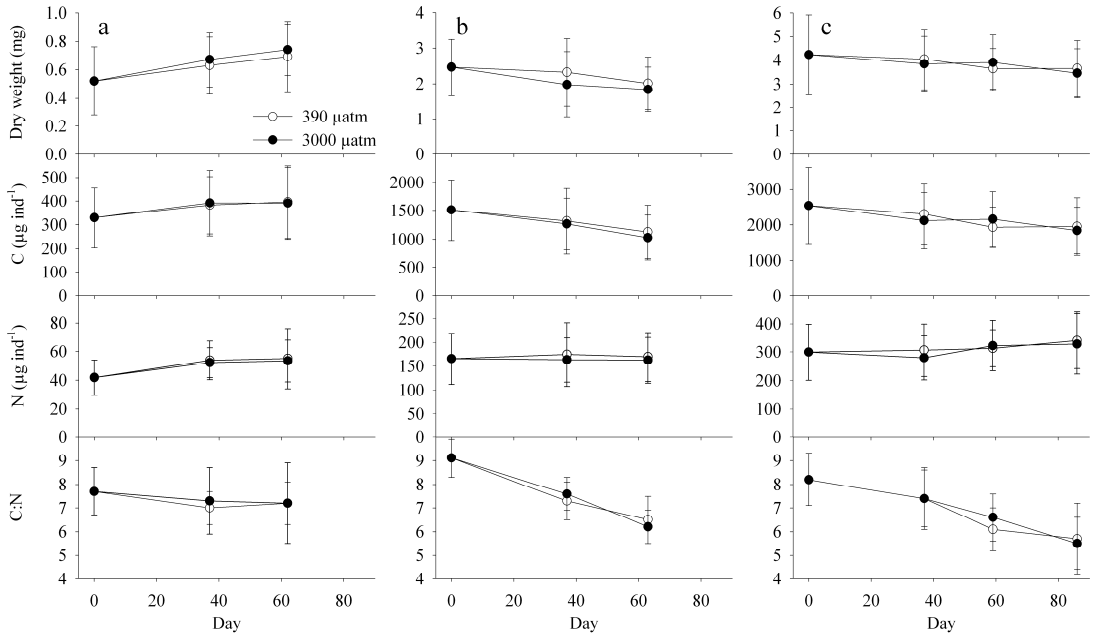

Fig. 1 Dry weight, carbon (C) and nitrogen (N) content and C:N ratio of *Calanus glacialis* CV (a), *C. hyperboreus* CV (b) and *C. hyperboreus* females (c) incubated at 0 °C and 390 (white circles) and 3000 µatm pCO_2 (black circles). Please note different scaling of the y axes.

Dry weight of both *C. hyperboreus* life stages decreased, which was due to a decrease in body C content by 24 - 33 % over the course of the incubation (SR, CV: $p = 0.010$ (control) and <0.001 (high pCO_2); females: $p = 0.041$ (control) and 0.033 (high pCO_2)). The N content remained constant, and therefore the C:N ratio decreased significantly in all groups (SR, $p < 0.001$).

3.1.2 Respiration rates

In general, respiration rates were not affected by high pCO_2 (Fig. 2). Only in *C. hyperboreus* CV on day 15 and in *C. glacialis* CV on day 37, the oxygen consumption was significantly lower in copepods kept at high pCO_2 than in copepods at control conditions (t-test, $p = 0.023$ (*C. hyperboreus* CV) and 0.003 (*C. glacialis* CV)). The mass-specific respiration rates for *C. hyperboreus* were 0.30 ± 0.11 and 0.33 ± 0.06 ml O_2 g dry weight^{-1} h^{-1} for CV and adult females, respectively. The smaller *C. glacialis* CV consumed 0.51 ± 0.13 ml O_2 g dry weight^{-1} h^{-1}.

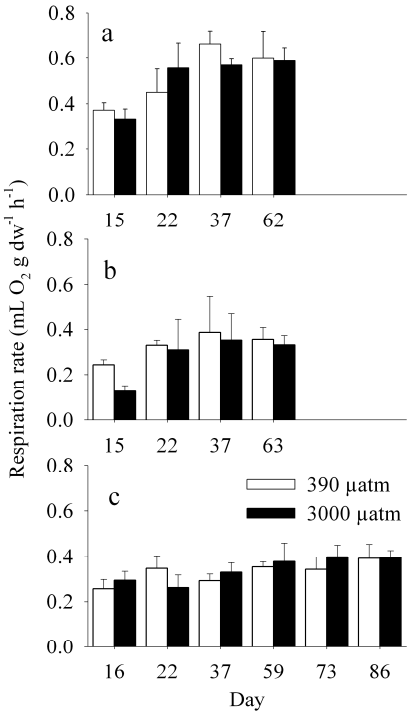

Fig. 2 Mass-specific respiration rates of *Calanus glacialis* CV (a), *C. hyperboreus* CV (b) and *C. hyperboreus* females (c) incubated at different pCO_2 (390: white bars; 3000 µatm: black bars). Significant differences between CO_2 treatments are marked with asterisks (*). dw = dry weight.

3.1.3 Gonad development

At the beginning of the experiment on 11 August, most *C. hyperboreus* females were semi-mature (GS 2 and 3; Fig. 3), while few individuals still had empty diverticula (GS

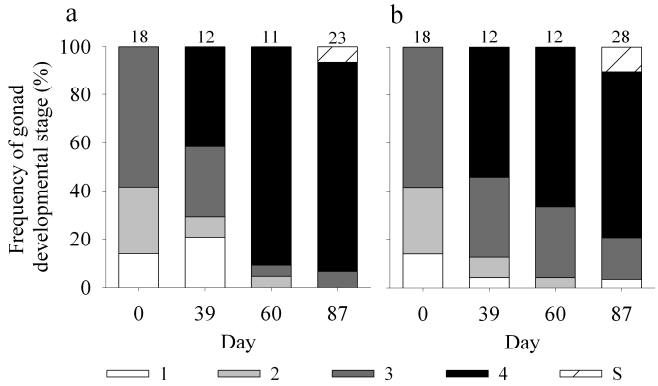

Fig. 3 Gonad development in *Calanus hyperboreus* females incubated at 390 µatm pCO_2 (a) and 3000 µatm pCO_2 (b). Developmental stages were determined after Hirche and Niehoff (1996) and Niehoff (1998). The number of females analyzed (n) is presented above the columns.

1). No mature females (GS 4) were present. In the following weeks, the proportion of mature females increased to 87 (control pCO_2) and 69 % (high pCO_2) in early November when the experiment was stopped (Fig. 3, 4). By this time, a small number of females was already spent, i.e. all eggs were laid (S). No significant difference in the gonad development was found between copepods incubated at control and high pCO_2.

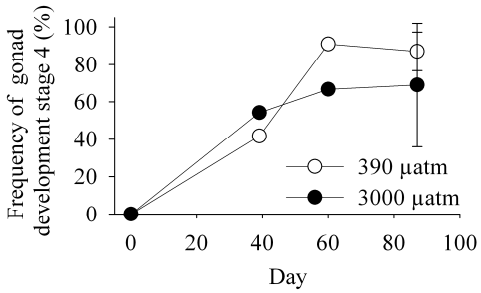

Fig. 4 Frequency of mature *Calanus hyperboreus* females (gonad development stage 4) during an incubation at 390 µatm (white circles) and 3000 µatm pCO_2 (black circles).

3.1.4 Mortality

Negative effects of elevated pCO_2 on the survival of the copepods were not detected (Fig. 5). In *C. glacialis* CV 0.7 ± 0.1 % copepods day^{-1} died, adding up to 43 ± 8 % of the copepods initially incubated. In *C. hyperboreus* CV, 0.6 ± 0.2 % individuals day^{-1} died (39 ± 9 % in total). In *C. hyperboreus* females mortality was slightly lower with 0.5 ± 0.1 % copepods day^{-1} (40 ± 7 % in total).

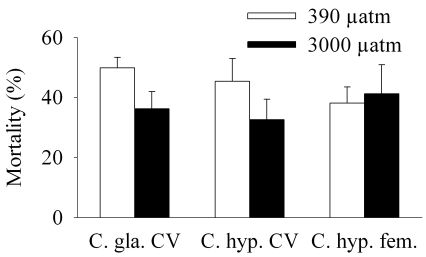

Fig. 5 Mortality (% of initial number of copepods) of *Calanus glacialis* CV (C. gla. CV), *C. hyperboreus* CV (C. hyp. CV) and *C. hyperboreus* females (C. hyp. fem.) after 62, 63 and 86 days of incubation, respectively. White bars: 390 µatm pCO_2; black bars: 3000 µatm pCO_2. Significant differences between CO_2 treatments are marked with asterisks.

3.2 Effects of elevated pCO_2 and temperature on *Calanus hyperboreus* females

In 2011, we studied the combined effects of elevated pCO_2 and temperature (0, 5 and 10 °C) using female *C. hyperboreus* due to their longevity during our first experiment. Incubating the females at 0 °C, which is close to the temperature during overwintering in nature, also allowed to compare the responses to pCO_2 in females captured in different years. Although females in 2011 were smaller than that captured in 2010, their responses to 390 and 3000 µatm in terms of decrease in body mass, specific respiration rates and gonad development was the same in the two years, indicating that the results gained during the first experiment are well reproducible.

3.2.1 Body mass development

C. hyperboreus females (prosome length 6.5 ± 0.4 mm) had a dry weight of 3.59 ± 1.36 mg, a C content of 2002 ± 937 µg and an N content of 261 ± 79 µg at the start of the incubation experiment. Dry weight decreased significantly during the incubation (SR, $0.001 < p \leq 0.013$), as did the carbon content (SR, $0.001 < p \leq 0.008$) (Fig. 6). There were, however, differences in the relative losses of C among the different CO_2 and temperature treatments, and also the nitrogen content did not develop consistently.

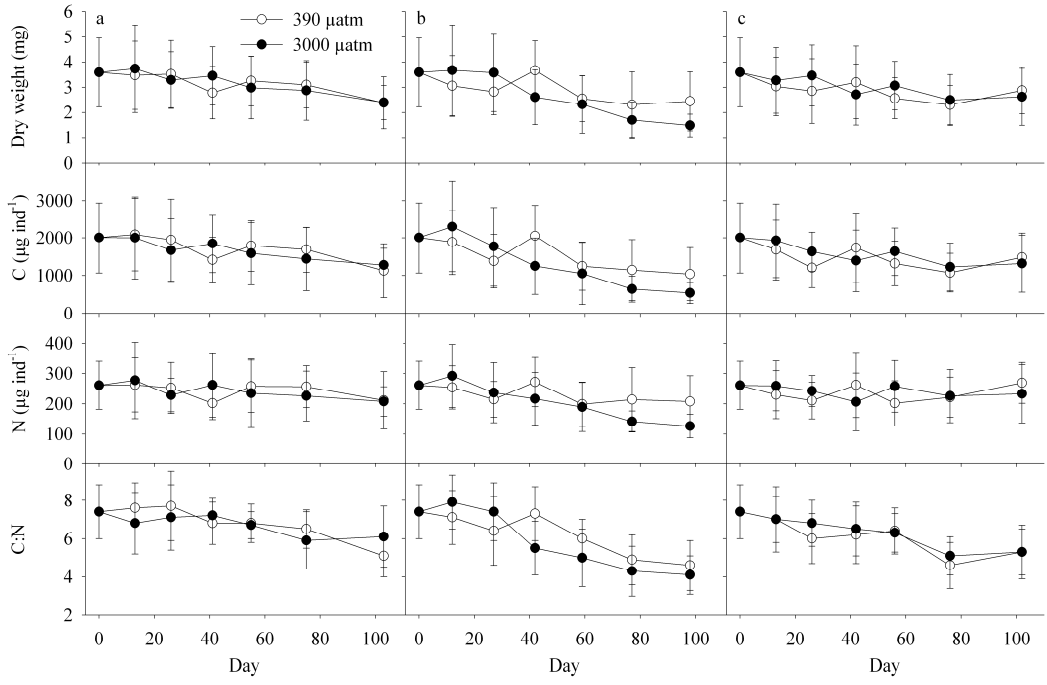

Fig. 6 Dry weight, carbon (C) and nitrogen (N) content and C:N ratio of *Calanus hyperboreus* females incubated at 0 °C (a), 5 °C (b) and 10 °C (c) at different pCO_2 (390 µatm: white circles; 3000 µatm: black circles).

At 0 °C, the body C decreased by 44 % at control condition and 36 % at high pCO_2. The nitrogen content remained constant at control condition and decreased significantly at high pCO_2 (SR, p = 0.038). At 5 °C, the body C decreased by 48 % at 390 µatm CO_2 and by 73 % C at 3000 µatm CO_2. The N content also decreased significantly at control condition (27 %) and high pCO_2 (52 %) (SR, p = 0.015 and < 0.001, respectively). In females incubated at 10 °C, the C content decreased by 26 % (control) and 34 % (high pCO_2). The N content did not change significantly over time in both groups.

The total amount of body C and N of the copepods after 15 weeks of incubation was significantly dependent on temperature and, for N, also on pCO_2 (two-way ANOVA, p ≤ 0.035, Table 4). Post-hoc Holm-Sidak tests revealed that the C and N contents of copepods incubated at 5 °C and high pCO_2 were significantly lower as compared to copepods from all other treatments, indicating that only the combination of an increase in temperature to 5 °C and high pCO_2 had an effect on the copepod body mass (Fig. 6).

3.2.2. Respiration rates

At all three temperatures, there was no significant difference between the respiration rates of *C. hyperboreus* females kept at 390 and 3000 µatm CO_2. The respiration rates at the three temperatures, however, were significantly different (two-way ANOVA, p < 0.001; Table 4, Fig. 7).

Table 4 Two-way ANOVA results for the effect of elevated seawater pCO_2 and temperature on *Calanus hyperboreus* females.

		DF	SS	MS	F	P
Dry weight	CO_2	1	2.820	2.820	3.236	0.077
	Temperature	**2**	**7.296**	**3.648**	**4.187**	**0.019**
	CO_2 * Temperature	2	3.098	1.549	1.777	0.177
C content	CO_2	1	0.103	0.103	1.846	0.179
	Temperature	**2**	**0.966**	**0.483**	**8.634**	**<0.001**
	CO_2 * Temperature	2	0.335	0.167	2.993	0.058
N content	**CO_2**	**1**	**26502.985**	**26502.985**	**4.674**	**0.035**
	Temperature	**2**	**78115.064**	**39057.532**	**6.888**	**0.002**
	CO_2 * Temperature	2	16219.832	8109.916	1.430	0.247
C:N	CO_2	1	0.001	0.001	0.098	0.755
	Temperature	**2**	**0.160**	**0.080**	**6.705**	**0.002**
	CO_2 * Temperature	2	0.043	0.022	1.822	0.171
Respiration	CO_2	1	0.181	0.181	2.406	0.122
	Temperature	**2**	**8.575**	**4.287**	**56.988**	**<0.001**
	CO_2 * Temperature	2	0.344	0.172	2.286	0.103

Significant effects are marked in **bold**.

At 0 °C, the respiration rates ranged between 0.26 ± 0.03 and 0.46 ± 0.04 (overall mean = 0.35 ± 0.14) ml O_2 g dry weight^{-1} h^{-1} and did not change significantly over the course of the experiment (Fig. 7a). In females kept at 5 °C the oxygen consumption was almost twice as high with 0.66 ± 0.15 ml O_2 g dry weight^{-1} h^{-1} over the entire incubation (Fig. 7b). At 10 °C, the oxygen consumption of *C. hyperboreus* females was higher by a factor of three (1.25 ml O_2 g dry weight^{-1} h^{-1}) three days after the temperature was raised. During the following weeks, the respiration rates decreased and for the last two months of the experiment, they were only slightly higher than in copepods kept at 0 °C (Fig. 7c).

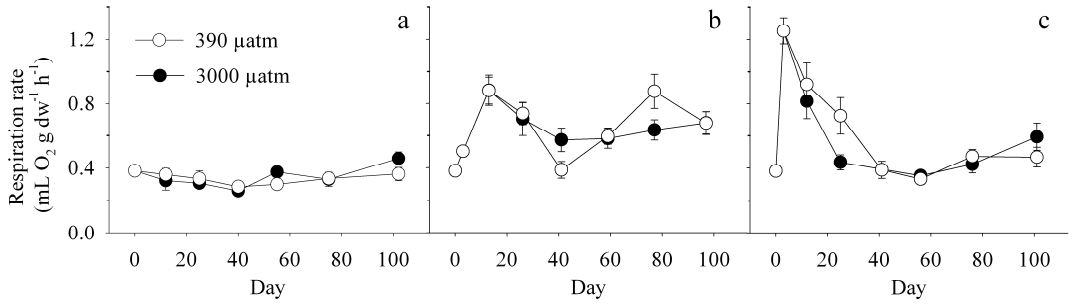

Fig. 7 Mass-specific respiration rates of *Calanus hyperboreus* females incubated at 0 °C (a), 5 °C (b) and 10 °C (c) at different pCO_2 (390 µatm: white bars; 3000 µatm: black bars). Rates are presented as mean ± standard error. The rate at day 0 presents the measurement before manipulation of pCO_2 and temperature. Rates at day 3 were measured after increasing the temperature. CO_2 was added on day 5. dw = dry weight.

3.2.3 Gonad development

The gonad development was not significantly influenced by pCO_2, except for week six, when the proportion of mature females at 10 °C was significantly higher in copepods kept a high as compared to control CO_2 (Fig. 8, 9). Differences were, however, found in females kept at different temperatures.

At the beginning of the incubation, 19 August, all females were immature (GS 1) or semi-mature (GS 2 and GS 3). At 0 °C, the number of mature females (GS 4) increased over the incubation, reaching 71 (control pCO_2) and 91 % (high pCO_2) by the end of November (Fig. 8a, 9a). At 5 °C, the frequency of mature females increased during the first six (83 %, high CO_2) to nine weeks (88 %, control CO_2) of the experiment. Thereafter, the number of senescent females increased and 30 (control pCO_2) and 54 % (high pCO_2) of the females were spent (S) at the end of the experiment (Fig. 8b, 9b).

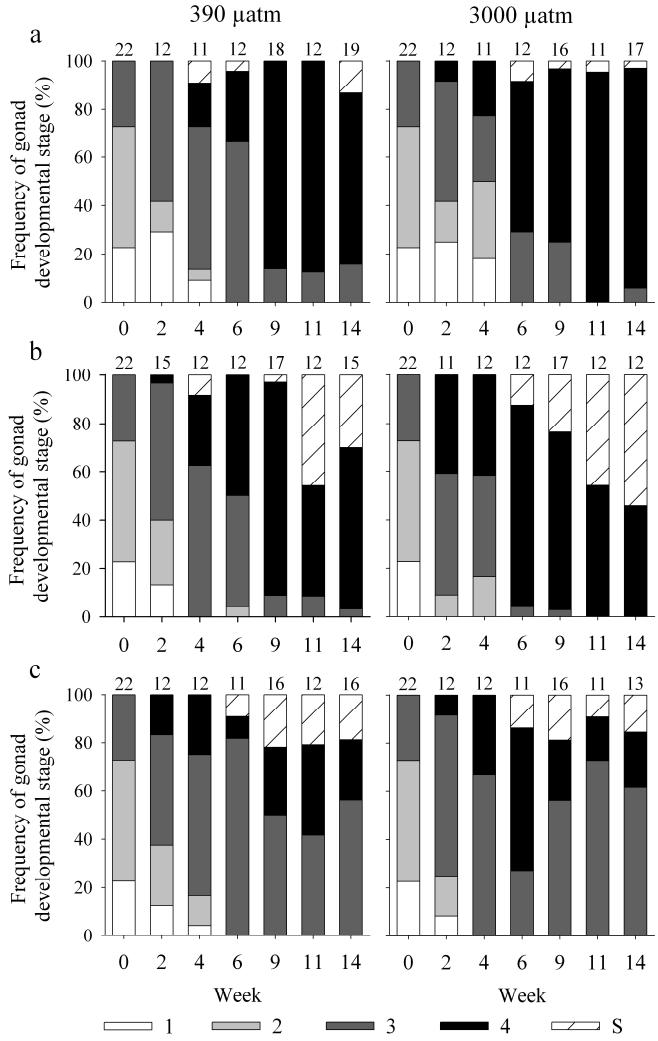

Fig. 8 Gonad development in *Calanus hyperboreus* females incubated at 0 °C (a), 5 °C (b) and 10 °C (c) at 390 (left side) and 3000 µatm pCO_2 (right side). Developmental stages were determined after Hirche and Niehoff (1996) and Niehoff (1998). The number of females analyzed (n) is presented above the columns.

Significant differences in the proportion of mature and senescent females were found between copepods from 0 and 5 °C during the last three weeks of the incubation (z-tests; 390 µatm: week 11, S: $p = 0.029$; 3000 µatm: week 14/15, GS 4: $p = 0.023$, S: $p = 0.006$). In *C. hyperboreus* incubated at 10 °C, mature females were less frequent throughout the experiment (< 59 %) and made up a significant lower proportion as compared to those from 0 °C during the last 6 weeks of the incubation (z-test, $0.001 \leq p \leq 0.035$). Most females (56 % at control pCO_2, 62 % at high pCO_2) at 10 °C were in a semi-mature state (GS 3) at the end of the experiment. Accordingly, also the number of senescent females was low (Fig. 8c, 9c).

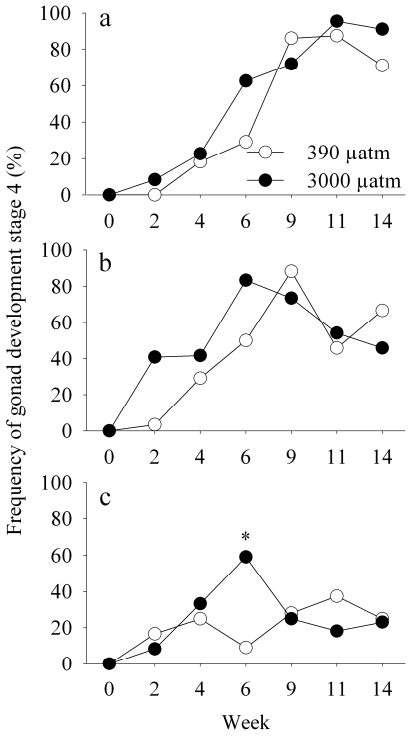

Fig. 9 Frequency of mature *Calanus hyperboreus* females (gonad development stage 4) during incubations at 0 °C (a), 5 °C (b) and 10 °C (c) at 390 µatm (white circles) and 3000 µatm pCO_2 (black circles). Significant differences between CO_2 treatments are marked by asterisks (*).

3.2.4 Mortality

The mortality during the incubation was not influenced by the experimental conditions, i.e. temperature, CO_2 and the combination of both factors (Fig. 10). It was 0.14 ± 0.08 % copepods day^{-1} and thus lower as compared to the experiment in 2010. Only at 10 °C and 390 µatm CO_2, an exceptionally high number of females (11 out of 40) died between day 3 and day 5 in one of the four incubation bottles that were run simultaneously. After 17 weeks of incubation, the total mortality added up to 15 ± 7 %

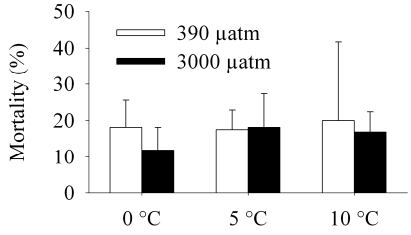

Fig. 10 Mortality (% of initial number of copepods) of *Calanus hyperboreus* females incubated at three different temperatures (0, 5, 10 °C) after 119 days of incubation. White bars: 390 µatm pCO_2; black bars: 3000 µatm pCO_2.

(0 °C), 18 ± 7 % (5 °C) and 18 ± 15 % (10 °C) of the *C. hyperboreus* females that were initially incubated.

4 DISCUSSION

4.1 Copepod condition during the experiments

Up to date, the impact of elevated pCO_2 on calanoid copepod species has been investigated in short-term laboratory and mesocosm studies that did not exceed 30 days, and all studies have been performed with copepods that were feeding and/or reproducing (e.g. Kurihara et al. 2004, Mayor et al. 2007, Zhang et al. 2011, Niehoff et al. 2013). A striking life history trait of Arctic *Calanus* species, however, is that they spend the winter at greater depth in a resting state (diapause, for reviews see Smith and Schnack-Schiel 1990, Dahms 1995, Hirche 1996) and there is no information on how the copepods respond to elevated pCO_2 and/or temperatures at that time. Factors triggering the diapause are not yet fully understood (e.g. Johnson et al. 2008, Ji 2011), and as the animals were still feeding when we captured them in July we have chosen to continuously provide food during the first incubation from mid August until mid November 2010. During our experiment, *C. glacialis* CV fed readily on the diatom *Thalassiosira weissflogii* (pers. obs.) and carbon and nitrogen content increased, indicating that *C. glacialis* assimilated the dietary compounds. Also, the respiration rates were in the same range of that of feeding *C. glacialis* CV in spring (Båmstedt and Tande 1985). We therefore conclude that *C. glacialis* did not enter diapause and that the data reflect the long-term response of active individuals of this species to ocean acidification.

Also *C. hyperboreus* is known to feed on *T. weissflogii* during experiments in spring and summer (Graeve et al. 2005, Niehoff, pers. obs.). During our first incubation in 2010, however, which started in August when copepods in the field descend to deeper waters (Dawson 1978, Hirche 1997), *C. hyperboreus* CV and females did not ingest the algae, which we provided, and lost significant amounts of body carbon. This suggests that both, CV and females had entered the overwintering resting stage, during which they rely on internal lipid reserves only. During the second incubation experiment in 2011, we therefore focused on *C. hyperboreus* females to investigate the response of a diapausing copepod to elevated pCO_2 and temperatures. In addition, their mortality had

been the lowest of the three groups incubated in 2010 and this allowed to extending the incubation to December.

4.2 Tolerance of *Calanus glacialis* and *C. hyperboreus* to elevated pCO_2 during long-term exposure

In our study, we did not find any indication of an influence of pCO_2 on copepods kept at 0 °C, which is close to the *in situ* temperature during winter. There were no differences in the response between the CV of the two species and between the CV and the females of *C. hyperboreus*. The two *Calanus* species from the Fram Strait were thus tolerant to elevated pCO_2 and likely late developmental stages will not suffer directly from CO_2 levels which are projected for the end of 2300 (e.g. Caldeira and Wickett 2003). It is, however, possible that earlier copepodites and nauplii may not be able to tolerate high pCO_2 as Kurihara et al. (2004) have shown that the mortality of nauplii of *Acartia erythraea* increased at approx. 5000 and 10,000 µatm whereas adults tolerated such conditions. Ocean acidification can also indirectly influence the copepods when CO_2 alters the food availability and quality (Rossoll et al. 2012, de Kluijver et al. 2013), thus studies on feeding biology and trophic interactions in an acidified ocean are necessary.

Elevated pCO_2 could have altered the metabolism of the copepods in different ways. In the sipunculid worm *Sipunculus nudus*, the jumbo squid *Dosidicus gigas* and the pteropod *Limacina helicina antarctica*, the oxygen consumption rates decreased at high pCO_2 (Reipschläger and Pörtner 1996, Rosa and Seibel 2008, Seibel et al. 2012), whereas they increased in the calanoid copepod *Centropages tenuiremis* (Li and Gao 2012). In our study, in contrast, the respiration rates did not differ between copepods kept at 3000 µatm pCO_2 and control conditions. Also the body mass was similar in the two groups, suggesting that the energy budget was not influenced by elevated pCO_2. The survival rates of the copepods were also not negatively affected by a pCO_2 of 3000 µatm over several months. Previous studies revealed that female *C. glacialis*, *C. sinicus*, *Acartia steueri* and *A. pacifica* may survive even extremely high pCO_2 of 10,000 µatm (pH = 6.9), however, these studies lasted for only approx. one week (Kurihara et al. 2004, Zhang et al. 2011, Weydmann et al. 2012).

Gonad development was only studied in female *C. hyperboreus* as their gonads develop and females spawn from mid September until February in the Fram Strait (Hirche and

Niehoff 1996). It was therefore possible to compare the timing of appearance and the proportion of mature females between individuals kept at different pCO_2. To keep the stress for the females at minimum, we staged the gonads only when we sorted individuals for respiration and body mass measurements and the intervals were thus large with 1-2 weeks. On this rough scale, we did not find any differences in the gonad development rate between control and high pCO_2 treatments suggesting that the oocyte maturation processes were not severely affected. Other parameter characterizing reproductive success such as egg production and hatching rates were not measured. However, most studies on calanoid copepods yet published indicate that reproduction is only impaired at a $pCO_2 \geq 5000$ µatm (Kurihara et al. 2004, Mayor et al. 2007, Weydmann et al. 2012, McConville et al. 2013). Only in *C. tenuiremis,* egg production and hatching rates decreased and naupliar mortality increased already at 2000 µatm pCO_2 (Zhang et al. 2011). A $pCO_2 > 2000$ µatm in the seawater is not likely associated with climate change (Caldeira and Wickett 2003), but may be reached when CO_2 will be sequestered in the deep ocean to remove it from the atmosphere (e.g. Herzog et al. 1996, Adams and Caldeira 2008). *C. hyperboreus*, although epipelagic during spring and summer, reproduces at depth during winter (e.g. Smith 1990, Hirche 1991, Hirche and Niehoff 1996) and egg production and hatching success of this species may then indeed be threatened by high pCO_2.

4.3 Warming could alter OA effects

Recent studies have shown that in several marine invertebrate taxa a combination of OA and ocean warming may lead to synergistic effects on e.g. growth, fertilization and thermal tolerance that are stronger than the additive response to both stressors alone would indicate (e.g. Metzger et al. 2007, Findlay et al. 2010, Ericson et al. 2012, Albright and Mason 2013). On copepods, yet only three laboratory studies investigated the combined effects of elevated temperature and CO_2 indicating that the response differs among species. Vehmaa et al. (2012) compared the reproduction of *Acartia bifilosa* at 17 and at 20 °C and at a pH of ~8.1 and ~7.5 and found significant synergistic effects on the total egg and nauplii production after five days of incubation. In contrast, egg hatching rates in *C. helgolandicus* were not affected by a combination of OA (pH of ~7.7) and elevated temperatures (ΔT = 2-4 °C) after a three days exposure (Mayor et al. 2012). Zervoudaki et al. (2014) just recently showed that elevated pCO_2 (824 µatm) and temperatures (ΔT = 4 °C) did not affect egg production, hatching and

respiration of *A. clausi*. Only the excretion had increased at high pCO_2 and temperature but cause and consequences remain unknown (Zervoudaki et al. 2014).

In our long-term study, diapausing *C. hyperboreus* females exposed to 5 °C and 3000 µatm CO_2 lost significantly more carbon and nitrogen as compared to all other treatment groups. Other authors discuss that the energetic costs for acid-base regulation (Li and Gao 2012) and reproduction (Veehma et al. 2012) in copepods increase at exposure to elevated CO_2. The high loss in body mass we found could mirror such increase in metabolic needs. However, it should have been accompanied by higher respiration rates but no significant differences were found in oxygen consumption between females at 390 and 3000 ppm. In fact the mean rates were almost the same in these two groups, except for only one day (day 25), and we cannot explain the combined CO_2/temperature effect on body mass we observed at 5 °C. At 10 °C, adding CO_2 as a stressor in addition to temperature did not change the performance of *C. hyperboreus* females. Here, the temperature stress and the decrease in metabolic rates might have outweighed possible synergistic effects of increased pCO_2 and temperatures.

4.4 Major effects of temperature on *Calanus hyperboreus* females

C. hyperboreus experiences a wide temperature range from -1.8 °C in the Arctic ocean to 8 °C in the Gulf of Maine (Conover and Corner 1968, Hirche and Niehoff 1996). However, in the cold deep waters where it spends the winter-months, the variation is usually low and temperatures never reach 5 or 10 °C as in our incubation experiment (Conover 1962, Falk-Petersen et al. 2009, Klages and Rohardt 2011). Such high temperatures over 17 weeks did not affect the survival of *C. hyperboreus* females, however, their metabolic rates changed.

During our incubation experiment in 2011, the respiration rates at 0 °C ranged between 0.26 and 0.46 ml O_2 g dry weight^{-1} h^{-1}. This is similar to respiration rates previously found in females from the Fram Strait at similar temperatures (Auel et al. 2003: 0.26 to 0.43 ml O_2 g dry weight^{-1} h^{-1}; Hirche 1987: 0.3-0.38 ml O_2 g dry weight^{-1} h^{-1}). Mature females were observed from mid September on and their number increased until the end of the experiment in December. This matches the reproductive cycle as described in a laboratory study with females from the Greenland Sea Basin (Hirche and Niehoff 1996).

During our incubation, the females lost significant amounts of body C (44 %) and the C:N ratio decreased. This indicates that the females relied on internal lipid reserves for fueling basic metabolism and reproduction as they do in nature (e.g. Conover and Corner 1968, Hirche and Niehoff 1996, Plourde et al. 2003).

When we exposed the females to 5 °C, their oxygen consumption doubled (0.66 ml O_2 g dry weight^{-1} h^{-}1) and remained at a high level over the entire experiment. The females lost 48 % body C, which was only slightly higher and not significantly different from that of females kept at 0 °C. Also in this group, the C:N ratio decreased. Models have shown that the lipid store of *C. finmarchicus* is depleted earlier at higher temperatures and thus the diapause may be shorter than at lower temperatures (Saumweber und Durbin 2006, Pierson et al. 2013). Our experiment with *C. hyperboreus* does not suggest a similar mechanism. However, the number of individuals was limited in our study, and therefore we used only ten to twelve females in each group to determine final body C and N. Given that the individual variability was high, this number was likely not sufficient to detect significant differences in carbon content. It is also possible that the relatively low loss in carbon despite high respiration rates at 5 °C can be explained by a decrease in total fecundity at higher temperatures as Plourde et al. (2003) suggest. We only determined gonad development stages, and thus our experiment cannot clarify if the reproductive potential, i.e. the number of eggs produced during a female's lifetime, changed with temperature. However, our data suggest that the reproductive period of *C. hyperboreus* females ended earlier at 5 °C as compared to 0 °C, as a large proportion of females at 5 °C was already spent at the end of the experiment, while most females kept at 0 °C were mature and still spawning. Such shift in the reproductive period might severely affect the survival of the developing nauplii. As the reproduction of *C. hyperboreus* takes place during winter, the nauplii develop fueled by internal reserves until phytoplankton develops in spring (Conover 1967, Hirche and Niehoff 1996). Thus, if the reproduction were completed too early in winter, a temporal mismatch between the onset of feeding in the nauplii and the development of the spring bloom would impair the reproductive success of *C. hyperboreus*.

The loss of body C in copepods kept at 5 °C during our experiment was not only relatively low as compared to the copepods from 0 °C, but also when compared to *C. hyperboreus* females that were incubated at a similar temperature by Plourde et al.

(2003). Here, the copepods lost 81 % C during a 72-day experiment at 4.5 °C (Plourde et al. 2003). However, we kept the females in 5 L bottles and we only disturbed them once a week for most of the experiment when the water was exchanged by inverse filtration and specimens were sorted for regular measurements. Plourde et al. (2003), in contrast, aimed at measuring egg production rates and therefore kept the females in 50 mL petri dishes to count eggs and determine gonad development stage every two to three days. It is thus possible that the higher body carbon loss during their experiment may in part be attributed to more stress due to handling.

At 10 °C, the oxygen consumption reached a maximum of 1.25 ml O_2 g dry weight^{-1} h^{-1} but only for a short period (3 days) after increasing the water temperature to experimental conditions. Then, the oxygen consumption decreased and fell below that measured at 5 °C after three weeks. The mortality at 10 °C, however, was as low as that of females kept at 0 and 5 °C. This suggests that a temperature of 10 °C induces sublethal stress in *C. hyperboreus* females while temperatures > 15 °C have been shown to be lethal in a short-term experiment (Hirche 1987). Frederich and Pörtner (2000) have introduced a concept of oxygen-limited thermal tolerance, which proposes that temperatures above the species-specific optima lead to a decreased O_2 partial pressure in the hemolymph of ectothermic animals. This decrease in aerobic scope does not yet influence the survival, but limits the energy available for e.g. growth and reproduction (Pörtner 2001). Our data on the response of *C. hyperboreus* females to a temperature of 10 °C match this concept. Compared to copepods kept at 0 and 5 °C, gonad maturation was significantly delayed or even depressed under this condition, and only a small proportion of mature females was present at the end of our incubation. The total number of eggs released during the experiment was thus very likely low and this could explain why females at 10 °C lost less C as compared to copepods at 0 °C, despite higher respiration at the higher temperature.

5 CONCLUSION

Our experiments over several months with repeated measurements, including several aspects of the animal's ecology and physiology have shown that the subadult and adult life stages of the Arctic *Calanus* spp. we studied were robust to pCO_2 even above future levels of OA. Only synergistic effects of pCO_2 and temperature on body mass of *C.*

hyperboreus females found at 5 °C suggest sensitivity to climate change. Moreover, our study indicates that survival for several months is possible even at temperatures higher than that copepods in Fram Strait will experience during winter nowadays and in the next centuries. However, temperature likely impacts the reproductive period and the total reproductive output of *C. hyperboreus* females and therefore the population dynamics as previously discussed by Plourde et al. (2003). Temperatures rise not only in surface layers but also in deep Arctic waters (Soltwedel et al. 2005). Therefore, it is crucial to evaluate the effects of realistic temperature changes in combination with OA in future studies.

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REFERENCES

Adams, E. E. and Caldeira, K. (2008) Ocean storage of CO_2. *Elements* **4**, 319-324.

Albright, R. and Mason, B. (2013) Projected near-future levels of temperature and pCO_2 reduce coral fertilization success. *PLoS ONE* **8** (2), e56468. doi:10.1371/journal.pone.0056468.

Auel, H., Klages, M. and Werner, I. (2003) Respiration and lipid content of the Arctic copepod *Calanus hyperboreus* overwintering 1 m above the seafloor at 2,300 m water depth in the Fram Strait. *Mar. Biol*. **143**, 275-282.

Beszczynska-Möller, A. and Wisotzki, A. (2010) Physical oceanography during POLARSTERN cruise ARK-XXV/2. *Alfred Wegener Institute, Helmholtz Center for Polar and Marine Research*, Bremerhaven. doi:10.1594/PANGAEA.754250.

Båmstedt, U. and Tande, K. S. (1985) Respiration and excretion rates of *Calanus glacialis* in arctic waters of the Barents Sea. *Mar. Biol*. **87**, 259-266.

Caldeira, K. and Wickett, M. E. (2003) Anthropogenic carbon and ocean pH. *Nature* **425**, 365.

Conover, R. J. (1962) Metabolism and growth in *Calanus hyperboreus* in relation to its life cycle. *Rapp. Proc. Verb. Cons. Int. Expl. Mer* **153**, 190-197.

Conover, R. J. (1965) Notes on the molting cycle, development of sexual characters and sex ratio in *Calanus hyperboreus*. *Crustaceana* **8** (3), 308-320.

Conover, R. J.(1967) Reproductive cycle, early development, and fecundity in laboratory populations of the copepod *Calanus hyperboreus*. *Crustaceana* **13** (1), 61-72.

Conover, R. J. (1988) Comparative life histories in the genera *Calanus* and *Neocalanus* in high latitudes of the northern hemisphere. *Hydrobiologia* **167/168**, 127-142.

Conover, R. J. and Corner, E. D. S. (1968) Respiration and nitrogen excretion by some marine zooplankton in relation to their life cycles. *J. Mar. Biol. Ass. U.K.* **48**, 49-75.

Dahms, H.-U. (1995) Dormancy in the copepoda - an overview. *Hydrobiologia* **306**, 199-211.

Dawson, J. K. (1978) Vertical distribution of *Calanus hyperboreus* in the central Arctic Ocean. *Limnol. Oceanogr.* **23** (5), 950-957.

De Kluijver, A., Soetaert, K., Czerny, J., Schulz, K. G., Boxhammer, T., Riebesell, U. and Middelburg, J. J. (2013) A ^{13}C labeling study on carbon fluxes in Arctic plankton communities under elevated CO_2 levels. *Biogeosciences* **10**, 1425-1440.

Ericson, J. A., Ho, M. A., Miskelly, A., King, C. K., Virtue, P., Tilbrook, B. and Byrne, M. (2012) Combined effects of two ocean change stressors, warming and acidification, on fertilization and early development of the Antarctic echinoid *Sterechinus neumayeri*. *Polar Biol*. **35**, 1027-1034.

Fabry, V. J., Seibel, B. A., Feely, R. A. and Orr, J. C. (2008) Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES J. Mar. Sci*. **65**, 414-432.

Falk-Petersen, S., Pavlov, V., Timofeev, S. and Sargent, J. R. (2007) Climate variability and possible effects on Arctic food chains: The role of *Calanus*. In: Ørbæk, J. B., Kallenborn, R., Tombre, I., Hegseth, E. N., Falk-Petersen, S. and Hoel, A. H. (eds.) *Arctic alpine ecosystems and people in a changing environment.* Springer-Verlag, Berlin, Heidelberg, pp. 147-166.

Falk-Petersen, S., Mayzaud, P., Kattner, G. and Sargent, J. R. (2009) Lipids and life strategy of Arctic *Calanus*. *Mar. Biol. Res*. **5**, 18-39.

Findlay, H. S., Kendall, M. A., Spicer, J. I. and Widdicombe, S. (2010) Post-larval development of two intertidal barnacles at elevated CO_2 and temperature. *Mar. Biol.* **157**, 725-735.

Fitzer, S. C., Caldwell, G. S., Close, A. J., Clare, A. S., Upstill-Goddard, R. C. and Bentley, M. G. (2012) Ocean acidification induces multi-generational decline in

copepod naupliar production with possible conflict for reproductive resource allocation. *J. Exp. Mar. Biol. Ecol.* **418-419**, 30-36.

Frederich, M. and Pörtner, H. O. (2000) Oxygen limitation of thermal tolerance defined by cardiac and ventilator performance in spider crab, *Maja squinado*. A*m. J. Physiol. Regulatory Integrative Comp. Physiol*. **279**, 1531-1538.

Graeve, M., Albers, C. and Kattner, G. (2005) Assimilation and biosynthesis of lipids in Arctic *Calanus* species based on feeding experiments with a ^{13}C labelled diatom. *J. Exp. Mar. Biol. Ecol.* **317**, 109-125.

Grainger, E. H. (1961) The copepods *Calanus glacialis* Jaschnov and *Calanus finmarchicus* (Gunnerus) in Canadian Arctic-Subarctic waters. *J. Fish. Res. BD. Can*. **18** (5), 663-678.

Guillard, R. R. L. (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith, W. L. and Chanley, M. H. (eds.) *Culture of Marine Invertebrate Animals*. Plenum Press, New York, USA, pp. 26-60.

Hallberg, E. and Hirche, H.-J. (1980) Differentiation of mid-gut in adults and over-wintering copepods of *Calanus finmarchicus* (Gunnerus) and *C. helgolandicus* Claus. *J. Exp. Mar. Biol. Ecol*. **48**, 283-295.

Head, E. J. H. and Conover, R. J. (1983) Induction of digestive enzymes in *Calanus hyperboreus*. *Mar. Biol. Lett*. **4**, 219-231.

Head, E. J. H. and Harris, L. R. (1985) Physiological and biochemical changes in *Calanus hyperboreus* from Jones Sound NWT during the transition from summer feeding to overwintering condition. *Polar Biol*. **4**, 99-106.

Herzog, H. J., Adams, E. E., Auerbach, D. and Caulfield, J. (1996) Environmental impacts of ocean disposal of CO_2. *Energy Convers. Manag*. **37**, 999-1005.

Hirche, H.-J. (1987) Temperature and plankton II. Effect on respiration and swimming activity in copepods from the Greenland Sea. *Mar. Biol*. **94**, 347-356.

Hirche, H.-J. (1991) Distribution of dominant calanoid copepod species in the Greenland Sea during late fall. *Polar Biol*. **11**, 351-362.

Hirche, H.-J. (1996) The reproductive biology of the marine copepod, *Calanus finmarchicus* - a review. *Ophelia* **44**, 111-128.

Hirche, H.-J. (1997) Life cycle of the copepod *Calanus hyperboreus* in the Greenland Sea. *Mar. Biol.* **128**, 607-618.

Hirche, H.-J. (1998) Dormancy in three *Calanus* species (*C. finmarchicus*, *C. glacialis* and *C. hyperboreus*) from the North Atlantic. *Arch. Hydrobiol. Spec. Issues Advanc. Limnol*. **52**, 359-369.

Hirche, H.-J. and Mumm, N. (1992) Distribution of dominant copepods in the Nansen Basin, Arctic Ocean, in summer. *Deep-Sea Res*. **39** (Suppl. 2), 485-505.

Hirche, H.-J. and Niehoff, B. (1996) Reproduction of the Arctic copepod *Calanus hyperboreus* in the Greenland Sea – field and laboratory observations. *Polar Biol*. **16**, 209-219.

Ikeda, T., Torres, J. J., Hernández-León, S. and Geiger, S. P. (2000) Metabolism. In: Harris, R. P., Wiebe, P. H., Lenz, J., Skjoldal, H. R. and Huntley, M. (eds.) *ICES Zooplankton Methodology Manual*. Academic Press, London, UK and San Diego, CA, USA, pp. 455-532.

Jaschnov, W. A. (1970) Distribution of *Calanus* species in the seas of the northern hemisphere. *Int. Revue ges. Hydrobiol*. **55** (2), 197-212.

Ji, R. (2011) *Calanus finmarchicus* diapause initiation: new view from traditional life history-based model. *Mar. Ecol. Prog. Ser.* **440**, 105-114.

Johnson, C. L., Leising, A. W., Runge, J. A., Head, E. J. H., Pepin, P., Plourde, S. and Durbin, E. G. (2008) Characteristics of *Calanus finmarchicus* dormancy patterns in the Northwest Atlantic. *ICES J. Mar. Sci*. **65**, 339-350.

Kawaguchi, S., Kurihara, H., King, R., Hale, L., Berli, T., Robinson, J. P., Ishida, A., Wakita, M., Virtue, P., Nicol, S. and Ishimatsu, A. (2011) Will krill fare well under southern ocean acidification? *Biol. Lett.* **7**, 288-291.

Klages, M. and Rohardt, G. (2011) Physical oceanography during POLARSTERN cruise ARK-XXVI/2. *Alfred Wegener Institute, Helmholtz Center for Polar and Marine Research*, Bremerhaven. doi:10.1594/PANGAEA.78140.

Kurihara, H. and Ishimatsu, A. (2008) Effects of high CO_2 seawater on the copepod (*Acartia tsuensis*) through all life stages and subsequent generations. *Mar. Pollut. Bull*. **56**, 1086-1090.

Kurihara, H., Shimode, S. and Shirayama, Y. (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.

Kurihara, H., Matsui, M., Furukawa, H. and Hayashi, M. (2008) Long-term effects of predicted future seawater CO_2 conditions on the survival and growth of the marine shrimp *Palaemon pacificus*. *J. Exp. Mar. Biol. Ecol*. **367**, 41-46.

Lewis, C. N., Brown, K. A., Edwards, L. A., Cooper, G. and Findlay, H. S. (2013) Sensitivity to ocean acidification parallels natural pCO_2 gradients experienced by Arctic copepods under winter sea ice. *PNAS* **110** (51), E4960-E4967.

Lewis, E. and Wallace, D. W. R. (1998) CO2SYS - Program developed for the CO_2 system calculations. Carbon Dioxide Information Analysis Center, Report ORNL/CDIAC-105.

Li, W. and Gao, K. (2012) A marine secondary producer respires and feeds more in a high CO_2 ocean. *Mar. Pollut. Bull*. **64**, 699-703.

Longhurst, A. R. (1985) The structure and evolution of plankton communities. *Prog. Oceanogr*. **15**, 1-35.

Mayor, D. J., Matthews, C., Cook, K., Zuur, A. F. and Hay, S. (2007) CO_2-induced acidification affects hatching success in *Calanus finmarchicus*. *Mar. Ecol. Prog. Ser*. **350**, 91-97.

Mayor, D. J., Everett, N. R. and Cook, K. B. (2012) End of century ocean warming and acidification effects on reproductive success in a temperate marine copepod. *J. Plankton Res*. **34** (3), 258-262.

McConville, K., Halsband, C., Fileman, E. S., Somerfield, P. J. and Findlay, H. S. (2013) Effects of elevated CO_2 on the reproduction of two calanoid copepods. *Mar. Pollut. Bull.* **73** (2), 428-434.

Metzger, R., Sartoris, F. J., Langenbuch, M. and Pörtner, H. O. (2007) Influence of elevated CO_2 concentrations on thermal tolerance of the edible crab *Cancer pagurus*. *J. Therm. Biol*. **32**, 144-151.

Munday, P. L., Dixson, D. L., McCormick, M. I., Meekan, M., Ferrari, M. C. O. and Chivers, D. P. (2010) Replenishment of fish populations is threatened by ocean acidification. *PNAS* **107** (29), 12930-12934.

Niehoff, B. (1998) The gonad morphology and maturation in Arctic *Calanus* species. *J. Mar. Syst*. **15**, 53-59.

Niehoff, B., Schmithüsen, T., Knüppel, N., Daase, M., Czerny, J. and Boxhammer, T. (2013) Mesozooplankton community development at elevated CO_2 concentrations: results from a mesocosm experiment in an Arctic fjord. *Biogeosciences* **10**, 1391-1406.

Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., Key, R. M., Lindsay, K., Maier-Reimer, E., Matear, R., Monfray, P., Mouchet, A., Najjar, R. G., Plattner, G.-K., Rodgers, K. B., Sabine, C. L., Sarmiento, J. L., Schlitzer, R., Slater, R. D., Totterdell, I. J., Weirig, M.-F., Yamanaka, Y. and Yool, A. (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **437**, 681-686.

Pedersen, S. A., Hansen, B. H., Altin, D. and Osen, A. J. (2013) Medium-term exposure of the North Atlantic copepod Calanus finmarchicus (Gunnerus, 1770) to CO_2-acidified seawater: effects on survival and development. *Biogeosciences* **10**, 7481-7491.

Pierson, J. J., Batchelder, H., Saumweber, W., Leising, A. and Runge, J. (2013) The impact of increasing temperatures on dormancy duration in *Calanus finmarchicus*. *J. Plankton Res*. **35** (3), 504-512.

Plourde, S., Joly, P., Runge, J. A., Dodson, J. and Zakardjian, B. (2003) Life cycle of *Calanus hyperboreus* in the lower St. Lawrence Estuary and its relationship to local environmental conditions. *Mar. Ecol. Prog. Ser*. **255**, 219-233.

Pörtner, H. O. (2001) Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* **88**, 137-146.

Raven, J., Caldeira, K., Elderfield, H., Hoegh-Guldberg, O., Liss, P., Riebesell, U., Shepherd, J., Turley, C. and Watson, A. (2005) Ocean acidification due to increasing atmospheric carbon dioxide. *Policy document 12/05, The Royal Society*, UK.

Reipschläger, A. and Pörtner, H. O. (1996) Metabolic depression during environmental stress: the role of extracellular versus intracellular pH in *Sipunculus nudus*. *J. Exp. Biol*. **199**, 1801-1807.

Richardson, A. J. (2008) In hot water: zooplankton and climate change. *ICES J. Mar. Sci*. **65**, 279-295.

Riebesell, U., Zondervan, I., Rost, B., Tortell, P. D., Zeebe, R. E. and Morel, F. M. M. (2000) Reduced calcification of marine plankton in response to increased atmospheric CO_2. *Nature* **407**, 364-367.

Riebesell, U., Czerny, J., von Bröckel, K., Boxhammer, T., Büdenbender, J., Deckelnick, M., Fischer, M., Hoffmann, D., Krug, S. A., Lentz, U., Ludwig, A., Muche, R. and Schulz, K. G. (2013) Technical note: A mobile sea-going mesocosm system - new opportunities for ocean change research. *Biogeosciences* **10**, 1835-1847.

Rosa, R. and Seibel, B. A. (2008) Synergistic effects of climate-related variables suggest future physiological impairment in a top oceanic predator. *PNAS* **105**, 20776-20780.

Rossoll, D., Bermúdez, R., Hauss, H., Schulz, K. G., Riebesell, U., Sommer, U. and Winder, M. (2012) Ocean acidification-induced food quality deterioration constrains trophic transfer. *PLoS ONE* **7** (4), e34737. doi:10.1371/journal.pone.0034737.

Runge, J. A. (1988) Should we expect a relationship between primary production and fisheries? The role of copepod dynamics as a filter of trophic variability. *Hydrobiologia* **167/168**, 61-71.

Saumweber, W. J. and Durbin, E. G. (2006) Estimating potential diapause duration in *Calanus finmarchicus*. *Deep-Sea Res. II* **53**, 2597-2617.

Schnack-Schiel, S. B. and Isla, E. (2005) The role of zooplankton in the pelagic-benthic coupling of the Southern Ocean. *Sci. Mar*. **69** (Suppl. 2), 39-55.

Seibel, B. A., Maas, A. E. and Dierssen, H. M. (2012) Energetic plasticity underlies a variable response to ocean acidification in the pteropod, *Limacina helicina antarctica*. *PLoS ONE* **7** (4), e30464. doi:10.1371/journal.pone.0030464.

Smith, S. L. (1990) Egg production and feeding by copepods prior to the spring bloom of phytoplankton in Fram Strait, Greenland Sea. *Mar. Biol*. **106**, 59-69.

Smith, S. L. and Schnack-Schiel, S. B. (1990) Polar zooplankton. In: Smith Jr., W. O. (ed.) *Polar oceanography. Part B: Chemistry, biology, and geology*. Academic Press, New York, pp. 407-760.

Solomon, S., Quin, D., Manning, M., Alley, R. B., Berntsen, T., Bindoff, N. L., Chen, Z., Chidthaisong, A., Gregory, J. M., Hegerl, G. C., Heimann, M., Hewitson, B., Hoskins, B. J., Joos, F., Jouzel, J., Kattsov, V., Lohmann, U., Matsuno, T., Molina, M., Nicholls, N., Overpeck, J., Raga, G., Ramaswamy, V., Ren, J., Rusticucci, M., Somerville, R., Stocker, T. F., Whetton, P., Wood, R. A. and Wratt, D. (2007) Technical Summary. In: Solomon, S., Quin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M. and Miller, H. L. (eds.) *Climate Change 2007: The Physical Science Basis*. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Soltwedel, T., Bauerfeind, E., Bergmann, M., Budaeva, N., Hoste, E., Jaeckisch, N., von Juterzenka, K., Matthiessen, J., Mokievsky, V., Nöthig, E.-M., Quéric, N.-V., Sablotny, B., Sauter, E., Schewe, I., Urban-Malinga, B., Wegner, J., Wlodarska-Kowalczuk, M. and Klages, M. (2005) HAUSGARTEN. Multidisciplinary investigations at a deep-sea, long-term observatory in the Arctic Ocean. *Oceanography* **18** (3), 56-61.

Tande, K. S., Hassel, A. and Slagstad, D. (1985) Gonad maturation and possible life cycle strategies in *Calanus finmarchicus* and *Calanus glacialis* in the northwestern part of the Barents Sea. In: Gray, J. S. and Christiansen, M. E. (eds.) *Biology of polar regions and effect of stress on marine organisms.* J. Wiley & Sons Ltd., New York, pp. 141-155.

Vehmaa, A., Brutemark, A. and Engström-Öst, J. (2012) Maternal effects may act as an adaptation mechanism for copepods facing pH and temperature changes. *PLoS ONE* **7** (10), e48538. doi:10.1371/journal.pone.0048538.

Watanabe, Y., Yamaguchi, A., Ishida, H., Harimoto, T., Suzuki, S., Sekido, Y., Ikeda, T., Shirayama, Y., Takahashi, M. M., Ohsumi, T. and Ishizaka, J. (2006) Lethality of increasing CO_2 levels on deep-sea copepods in the western North Pacific. *J. Oceanogr*. **62**, 185-196.

Węsławski, J. M., Stempniewicz, L., Mehlum, F. and Kwaśniewski, S. (1999) Summer feeding strategy of the little auk (*Alle alle*) from Bjørnøya, Barents Sea. *Polar Biol*. **21**, 129-134.

Weydmann, A., Søreide, J. E., Kwasniewski, S. and Widdicombe, S. (2012) Influence of CO_2-induced acidification on the reproduction of a key Arctic copepod *Calanus glacialis*. *J. Exp. Mar. Biol. Ecol*. **428**, 39-42.

Wittmann, A. C. and Pörtner, H.-O. (2013) Sensitivities of extant animal taxa to ocean acidification. *Nat. Clim. Change* **3** (11), 995-1001.

Yamada, Y. and Ikeda, T. (1999) Acute toxicity of lowered pH to some oceanic zooplankton. *Plankton Biol. Ecol*. **46** (1), 62-67.

Zervoudaki, S., Frangoulis, C., Giannoudi, L. and Krasakopoulou, E. (2014) Effects of low pH and raised temperature on egg production, hatching and metabolic rates of

a Mediterranean copepod species (*Acartia clausi*) under oligotrophic conditions. *Mediterr. Mar. Sci*. **15** (1), 74-83.

Zhang, D., Li, S., Wang, G. and Guo, D. (2011) Impacts of CO_2-driven seawater acidification on survival, egg production rate and hatching success of four marine copepods. *Acta Oceanol. Sin*. **30** (6), 86-94.

PUBLICATION II

Effects of ocean acidification on grazing of *Calanus finmarchicus* and *C. glacialis* (Copepoda: Calanoida)

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ABSTRACT

It is currently under debate whether organisms that regulate their acid-base status under environmental hypercapnia demand additional energy. This could impair animal fitness, but might be compensated for via increased ingestion rates when food is available. No data are yet available for dominant *Calanus* spp. from Arctic waters. To fill this gap, we incubated *C. finmarchicus* and *C. glacialis* at 390, 1120 and 3000 µatm for about two weeks with *Thalassiosira weissflogii* (diatom) as food source on-board RV *Polarstern* in Fram Strait in 2012. Every three to four days copepods were sub-sampled from all CO_2 treatments and clearance and ingestion rates were determined. During the SOPRAN mesocosm experiment in Bergen, Norway, 2011, we weekly collected copepods from mesocosms initially adjusted to 390 and 3000 µatm CO_2 and also measured grazing at low and high pCO_2. In addition, copepods were deep frozen for body mass analyses. Elevated pCO_2 did not directly affect grazing activities and body mass, suggesting that the copepods did not have additional energy demands for coping with acidification, neither during long-term exposure nor after immediate changes in pCO_2. Compensating for shifts in seawater pH thus does not seem to challenge these copepods.

KEYWORDS: Ocean acidification • Ingestion rate • Clearance rate • Food uptake • *Calanus*

1 INTRODUCTION

Anthropogenic CO_2 emissions, which recently amounted to about 34 billion tons $year^{-1}$ (Friedlingstein et al. 2010), have increased the atmospheric CO_2 concentration from 280 to about 400 µatm since pre-industrial times (Tans 2013). About 30 % of the emitted CO_2 is absorbed by the world's oceans (Solomon et al. 2007), making them the second largest sink for human-made CO_2. The CO_2 uptake changes seawater chemistry resulting in decreasing seawater pH and carbonate ion concentrations (Raven et al. 2005). Model calculations based on the IPCC emissions scenario IS92a (Alcamo et al. 1995) projected that CO_2 concentrations might increase to about 750 µatm by 2100 and to almost 2000 µatm by the year 2300 (Caldeira and Wickett 2003), with a corresponding decline in surface ocean pH by about 0.4 units until the end of the century and by a maximum of 0.77 units until 2300, a process, usually referred to as "ocean acidification" (OA).

OA has the potential to severely affect the performance of marine organisms. Metabolic rates, for example, can be depressed at elevated CO_2 concentrations (Reipschläger and Pörtner 1996, Rosa and Seibel 2008, Seibel et al. 2012), possibly triggered by a lack of or incomplete compensation for increasing pCO_2 in extracellular fluids due to environmental hypercapnia (Reipschläger and Pörtner 1996, Pörtner et al. 1998). Food uptake and growth may then be impaired and mortality may increase (Michaelidis et al. 2005, Miles et al. 2007, Appelhans et al. 2012, Vargas et al. 2013). For species that are able to actively regulate their internal acid-base balance, such as crustaceans (Widdicombe and Spicer 2008), it is discussed that additional energy has to be allocated to maintaining the acid-base status, and therefore fitness is lowered at elevated seawater pCO_2 (Wood et al. 2008, Thomsen and Melzner 2010, Melzner et al. 2011, Appelhans et al. 2012, Saba et al. 2012). Accordingly, negative effects seem to be particularly distinct when food is limited (Melzner et al. 2011, Appelhans et al. 2012), while, when food supply is sufficient, feeding rates may increase to meet the additional energy demands, as suggested for krill (Saba et al. 2012) and the calanoid copepod *Centropages tenuiremis* (Li and Gao 2012).

Calanoid copepods are key players in pelagic marine environments and often dominate zooplankton communities (Longhurst 1985). Studies on the sensitivity of these

copepods to future OA show mixed responses. Levels of OA that are predicted to occur until 2300 were detrimental only in *Acartia tonsa* (Dupont and Thorndyke 2008), *A. spinicauda* and *C. tenuiremis* (Zhang et al. 2011). In all other species studied so far, parameters such as growth, egg production, hatching success and survival of nauplii, copepodites and adults were only affected at CO_2 concentrations ≥ 5000 µatm, if at all (e.g. Kurihara et al. 2004, Zhang et al. 2011, Mayor et al. 2012, McConville et al. 2013, Pedersen et al. 2013). Under controlled laboratory conditions, food uptake of calanoid copepods in response to elevated pCO_2 has only been studied in *C. tenuiremis* (Li and Gao 2012). Here, grazing rates decreased at elevated pCO_2 during the first day of exposure, however, no significant effects were found thereafter.

In our study, we focus on two *Calanus* species, i.e. *C. finmarchicus* (Gunnerus, 1770) and *C. glacialis* Jaschnov, 1955. Both are important components of the lipid-based food web of the Arctic (Falk-Petersen et al. 2007 and references therein). *C. finmarchicus* is a North Atlantic species and is transported into the Arctic Ocean via the West Spitsbergen Current, into the Barents Sea with the North Cape Current and into the Davis Strait with the West Greenland Current (e.g. Jaschnov 1970, Conover 1988). *C. glacialis* has its origin in the Arctic shelf regions and penetrates southward into the Fram Strait with the East Greenland Current (Jaschnov 1970, Conover 1988). In areas where the warm Atlantic water submerges under the cold Arctic water, both species co-occur (Conover 1988). During spring and summer, they inhabit surface waters where they feed on phytoplankton and accumulate large lipid stores (e.g. Marshall and Orr 1955, Lee 1974, Pasternak et al. 2001). In late summer they descend to deeper waters and enter a diapause to sustain the food scarce period in winter (e.g. Tande et al. 1985, Hirche 1998).

Previous studies on the response of *C. finmarchicus* and *C. glacialis* to OA indicate that copepodites V (CV) and females are relatively robust to CO_2 levels predicted for the next centuries (Mayor et al. 2007, Weydmann et al. 2012, Niehoff et al. 2013, Hildebrandt et al. in press). Feeding has not been studied under controlled laboratory conditions yet, however, Pedersen et al. (2013) just recently published a study on *C. finmarchicus* that were raised from eggs to adults at CO_2 concentrations of 3300 to 9700 µatm and showed that fat contents in CV grown at control and elevated pCO_2 were

similar. We may thus hypothesize that the food uptake was not lowered by high CO_2 concentrations. It remains open, however, whether *C. finmarchicus* compensated for additional energy demands due to OA stress with an increase in grazing rates (Saba et al. 2012). At more realistic surface seawater CO_2 concentrations (≤ 1420 µatm), de Kluijver et al. (2013) examined trophic interactions during a 30-day mesocosm study in an Arctic fjord, using ^{13}C as a tracer. Their study indeed found reduced rates of ^{13}C incorporation in *Calanus* spp. in high pCO_2 mesocosms, indicating that grazing rates decrease with increasing pCO_2. However, as the food quality, i.e. the algal community composition in the mesocosms, changed with the CO_2 concentration (Brussaard et al. 2013, Schulz et al. 2013), the decrease in grazing could reflect indirect effects of OA.

To elucidate whether *Calanus* spp. ingest more food to compensate for additional energy demands or whether ingestion rates decrease at OA, we performed controlled laboratory experiments at different pCO_2, feeding the copepods with monoalgal food (*Thalassiosira weissflogii*). Part of our study was conducted within the SOPRAN 2011 mesocosm experiment in Bergen, Norway. Here, we weekly sampled *C. finmarchicus* from mesocosms initially adjusted to 390 and 3000 µatm CO_2 and exposed the copepods of each group to both, high and low pCO_2 conditions to study immediate responses to changing pCO_2 as well as long-term effects of exposure to high pCO_2. In addition, we incubated *C. finmarchicus* and *C. glacialis* at 390, 1120 and 3000 µatm CO_2 for up to 16 days in the cold-rooms on-board RV *Polarstern* and repeatedly conducted grazing experiments.

2 METHODS

2.1 Experiments during the SOPRAN mesocosm study 2011

2.1.1 Sampling

In May and June 2011, experiments were conducted within the framework of the SOPRAN mesocosm experiment at Espegrend, the Marine Biological Station of the University of Bergen, Norway. Nine KOSMOS (Kiel Off-Shore Mesocosms for Future Ocean Simulations) mesocosms of 25 m length and 2 m diameter were deployed in the Raunefjord at N 60° 15.87', E 005° 12.33' for six weeks. For technical details on the mesocosms and their deployment, see Riebesell et al. (2013). Briefly, each mesocosm enclosed about 75 m^3 of fjord water containing natural plankton < 3 mm. Larger

mesozooplanktonic and nektonic organisms were excluded by a mesh (3 mm mesh size) that covered the openings of the mesocosm bags during deployment, as these organisms occur only in low numbers and are patchily distributed. On the lower end of each mesocosm, a sediment trap was installed to collect settling material. By adding CO_2 saturated fjord water to seven of the mesocosms in five steps over five days, the pH was adjusted to ~390, ~560, ~840, ~1120, ~1400, ~2000 and ~3000 µatm CO_2, while the remaining two mesocosms were kept at in-situ pCO_2 (~280 µatm). For our experiments, only copepods from the mesocosms with 390 (control) and 3000 µatm CO_2 (high pCO_2) were used. The pH (reported on the total scale) in the control mesocosm ranged between 8.0 and 8.1 throughout the experiment. In the high CO_2 mesocosm, the initial pH after CO_2 manipulation was ~7.2. However, due to outgassing and biological activities, it increased over time, especially in the upper water column. At the end of the experiment, the pH was 7.8 at the surface and 7.4 at depth. On day 14, nutrients (~5 µmol L^{-1} NO_3, ~0.16 µmol L^{-1} PO_4) were added to the mesocosms to induce a phytoplankton bloom.

Once a week zooplankton was sampled in each mesocosm with an Apstein net (mesh size 55 µm). Sampling depth was limited to 22 m in order not to resuspend material from the sediment traps. Within one hour, the plankton samples were brought to a cold room adjusted to 10 °C, which was the approximate in situ temperature at that time.

2.1.2 Preparation of experimental water and food algae

Filtered seawater was adjusted to 390 (control) and 3000 µatm CO_2 (high CO_2) by mixing with CO_2 saturated fjord water. The pH was monitored with a pH electrode (Mettler Toledo InLab Routine Pt1000, connected to a pH meter WTW pH 3310), which was calibrated with NIST buffers (pH 6.865 and 9.180). Then the pH was converted to total scale using TRIS-based reference material (Batch no. 4, A. Dickson, Scripps Institution of Oceanography). Mean pH values of control and high CO_2 treatments were 7.9 and 7.2, respectively (Table 1a). Temperature and salinity were determined with a conductivity meter WTW Cond340i (Table 1a). Monocultures of the diatom *T. weissflogii* were grown in f/2 medium (Guillard 1975) at 10 °C under constant light as food for the copepods.

Table 1 Water parameters during the determination of grazing rates (a) and during the CO_2 incubations on RV *Polarstern* in 2012 (b).

(a)

Species	CO_2 treatment (µatm)	Temp. (°C)	Salinity (psu)	pH (total scale)	Experiment
C. finmarchicus	390	10.0 ± 0.3	32.4 ± 0.2	7.86 ± 0.03	Bergen 2011
C. finmarchicus	3000	9.9 ± 0.2	32.4 ± 0.4	7.16 ± 0.07	Bergen 2011
C. finmarchicus	390	4.7 ± 0.1	34.1 ± 0.5	7.99 ± 0.03	RV *Polarstern* 2012
C. finmarchicus	1120	4.6 ± 0.1	34.2 ± 0.5	7.69 ± 0.05	RV *Polarstern* 2012
C. finmarchicus	3000	4.6 ± 0.1	34.4 ± 0.3	7.30 ± 0.07	RV *Polarstern* 2012
C. glacialis	390	1.1 ± 0.7	32.4 ± 1.2	7.97 ± 0.06	RV *Polarstern* 2012
C. glacialis	1120	1.0 ± 0.7	32.9 ± 0.5	7.62 ± 0.04	RV *Polarstern* 2012
C. glacialis	3000	1.0 ± 0.7	32.7 ± 0.7	7.21 ± 0.05	RV *Polarstern* 2012

(b)

Species	CO_2 treatment (µatm)	Temp. (°C)	Salinity (psu)	pH (total scale)
C. finmarchicus	390	4.5 ± 0.3	34.0 ± 0.4	8.01 ± 0.14
C. finmarchicus	1120	4.4 ± 0.2	34.3 ± 0.3	7.66 ± 0.11
C. finmarchicus	3000	4.4 ± 0.2	34.4 ± 0.2	7.29 ± 0.13
C. glacialis	390	1.4 ± 0.3	33.2 ± 0.4	7.86 ± 0.13
C. glacialis	1120	1.3 ± 0.4	33.1 ± 0.4	7.54 ± 0.09
C. glacialis	3000	1.3 ± 0.4	33.3 ± 0.4	7.21 ± 0.07

Values are presented as mean ± SD. Temp.: Temperature.

2.1.3 Determination of grazing rates

About 60 *Calanus finmarchicus* copepodites V (CV) were sorted from the control and high CO_2 mesocosm sample. To acclimate the copepods to laboratory conditions, they were then pre-incubated for one day at 10 °C in filtered seawater adjusted to the respective CO_2 concentration and containing *T. weissflogii*.

For the determination of grazing rates, high and control CO_2 water was inoculated with *T. weissflogii* at a concentration of 4000 cells mL^{-1}, yielding in initial chlorophyll *a* concentrations of 7.82 to 10.34 µg L^{-1}. 30 copepods from control and from high CO_2 mesocosms were transferred to 1 L bottles (10 copepods $bottle^{-1}$) containing seawater of the respective pCO_2. To test whether immediate changes in CO_2 concentration affect the food uptake, 30 copepods from the control mesocosm were placed in three 1 L bottles containing high CO_2 water, and 30 copepods from the high CO_2 mesocosm were transferred to 1 L bottles with control seawater. Three additional bottles for each CO_2 concentration were prepared that contained seawater with algae but no copepods. These

bottles were run as a blank to correct the grazing rates of the copepods for algal growth. All bottles were sealed airtight and mounted to a plankton wheel for 17.5 to 22 hours in the dark. Afterwards, the copepods were rinsed in distilled water and stored in tin caps at -20 °C to measure C and N content. Subsamples of 3x100 mL of the incubation water for each CO_2 treatment were filtered on GF/F filters and frozen to determine the chlorophyll *a* concentration prior to and at the end of the incubations.

2.2 On-board incubation experiments

2.2.1 Copepod sampling and incubation

In June and July 2012, experiments were conducted during the RV *Polarstern* cruises ARK-XXVII/1 and 2 to Fram Strait. Copepods were sampled with vertical bongo net hauls (200 and 300 µm mesh size; see Table 2) and immediately brought to a cooling container adjusted to 5 °C for *C. finmarchicus* and to 0 °C for *C. glacialis*. About 1800 *C. finmarchicus* CV and 1350 *C. glacialis* CV were sorted from the samples and acclimated to laboratory conditions in filtered seawater enriched with *T. weissflogii* for 2 days.

Table 2 Station list for copepod sampling in Fram Strait on-board RV *Polarstern*.

Date	Station	Position		Sampling depth (m)	Species
		Lat (degmin)	Lon (degmin)		
18.06.2012	PS 80/5-3	70° 59.18' N	08° 35.99' E	0-200	*C. finmarchicus*
03.07.2012	PS 80/91-2	79° 40.21' N	11° 59.64' W	0-250	*C. glacialis*
03.07.2012	PS 80/91-3	79° 40.32' N	11° 59.43' W	0-100	*C. glacialis*
03.07.2012	PS 80/91-4	79° 40.43' N	11° 59.22' W	0-100	*C. glacialis*
03.07.2012	PS 80/91-5	79° 40.55' N	11° 58.96' W	0-100	*C. glacialis*
03.07.2012	PS 80/91-6	79° 40.23' N	11° 59.42' W	0-100	*C. glacialis*
03.07.2012	PS 80/91-7	79° 40.34' N	11° 59.19' W	0-100	*C. glacialis*

Lat: Latitude, Lon: Longitude.

2.2.2 Preparation of incubation water and food algae

Filtered seawater was adjusted to 390 (control), 1120 (intermediate) and 3000 µatm (high) CO_2 by bubbling with a gas mixture from a gas cylinder. The resulting pH values (Table 1) differed at least by 0.3 units between control and intermediate and between intermediate and high CO_2 treatments. *T. weissflogii* was cultured according to the

experiment in 2011, but at temperatures of 10 and 18 °C. Before it was fed to the copepods, the algal suspension was adjusted to the respective incubation temperature.

2.2.3 CO_2 incubation

The copepods were transferred to 6 L glass bottles and incubated at control, intermediate and high CO_2 for 13 (*C. finmarchicus*) and 16 days (*C. glacialis*). Three bottles, each containing 200 *C. finmarchicus* and 150 *C. glacialis*, respectively, were set up for each CO_2 treatment. The copepods were fed daily with *T. weissflogii* at a concentration of 8000 cells mL^1 to prevent food limitation as these large bottles could not be mounted on a plankton wheel and therefore part of the algae sank to the bottom. Every three (*C. finmarchicus*) or four days (*C. glacialis*), the incubation water was changed and pH, salinity and temperature were measured. pH values increased on average by 0.14, 0.12 and 0.08 units in between water exchanges at 390, 1120 and 3000 µatm CO_2, respectively. When the water was changed, 10 copepods from each bottle were removed to determine grazing rates. At start and at the end of the incubations, 36 to 44 copepods from each CO_2 treatment were deep-frozen for C and N measurements. In addition, as we had sufficient numbers of *C. finmarchicus*, 12 individuals from every CO_2 concentration were removed from the incubation bottles during water exchanges to determine the C and N content.

2.2.4 Determination of grazing rates

30 copepods from each of the three different CO_2 treatments were transferred to three 1 L bottles (10 copepods $bottle^{-1}$) with seawater of the respective pCO_2. *T. weissflogii* was added at a concentration of 2000 cells ml^{-1}, which resulted in initial chlorophyll *a* concentrations of 2.5 to 6.2 µg L^{-1}. Along with three blanks for each CO_2 concentration (see above), the bottles were mounted to a plankton wheel for 20 to 25 hours. Subsamples for chlorophyll *a* measurements as well as copepod C and N samples were taken as described above.

2.3 Sample analysis

To calculate clearance and ingestion rates after Frost (1972), we determined the chlorophyll *a* concentration in the incubation bottles and blank bottles at the start and the end of the grazing experiments. The algal cells on the GF/F filters were first

disrupted in 90 % acetone using ultrasound (Branson Sonifier 250, Heinemann, Schwäbisch Gmünd, Germany). Then, the chlorophyll *a* was extracted from the algal cells for 2 hours in the dark at 5 °C. After centrifugation at 4500 rpm for 15 min at 0 °C, the chlorophyll *a* fluorescence in the supernatant was determined with a Turner fluorometer (TD-700, Turner Designs, Sunnyvale, CA, USA) at 665 nm before and after adding 2 drops of 1 N HCl.

When, in some treatments, the copepods did not feed at all or at very low rates, calculated grazing rates were negative. This indicates that the algae grew faster in bottles containing copepods as compared to the blanks without copepods, suggesting that the excretory products of the copepods have stimulated algal growth in the otherwise nutrient limited experimental bottles (Roman and Rublee 1980). Other studies have added f/2 to the bottles (e.g. Bartram 1980, Gentsch et al. 2009). However, with the addition of f/2, the pH of the seawater increased to 8 (personal observation) and the differences in pH between 390 and 3000 µatm diminished. We therefore did not add f/2 to our incubation bottles, even though grazing rates have likely been underestimated. Negative values were set to zero.

To determine carbon and nitrogen content, the copepods in the tin caps were dried at 60 °C for 48 hours and then analysed in an elemental analyser (Euro EA, HEKAtech GmbH, Wegberg, Germany). Acetanilide was used as standard.

2.4 Statistics

Statistical analyses were performed with SigmaStat 3.5 (Systat Software, Inc.). A t-test or a one-way ANOVA followed by a post-hoc Holm-Sidak test was performed to identify differences in grazing and body mass between copepods from different CO_2 treatments at each experimental day. When data were not normally distributed, a Kruskal-Wallis test followed by a post-hoc Tukey test was used instead. A Spearman Rank Order Correlation was used to identify changes in body carbon and nitrogen during the experiments. Data were considered significantly different at a $p < 0.05$. Results are presented as mean ± standard deviation.

3 RESULTS

In *C. finmarchicus* collected from mesocosms in the boreal Raunefjord, which were long-term exposed to control (390 µatm) and high pCO_2 (initially 3000 µatm), mean clearance rates (CR) ranged from 1.91 to 3.21 mL copepod^{-1} h^{-1} (Fig. 1A), and mean ingestion rates (IR) ranged from 0.007 to 0.018 µg chlorophyll *a* copepod^{-1} h^{-1} (Fig. 1B). Significant differences between copepods from high and control CO_2 were only

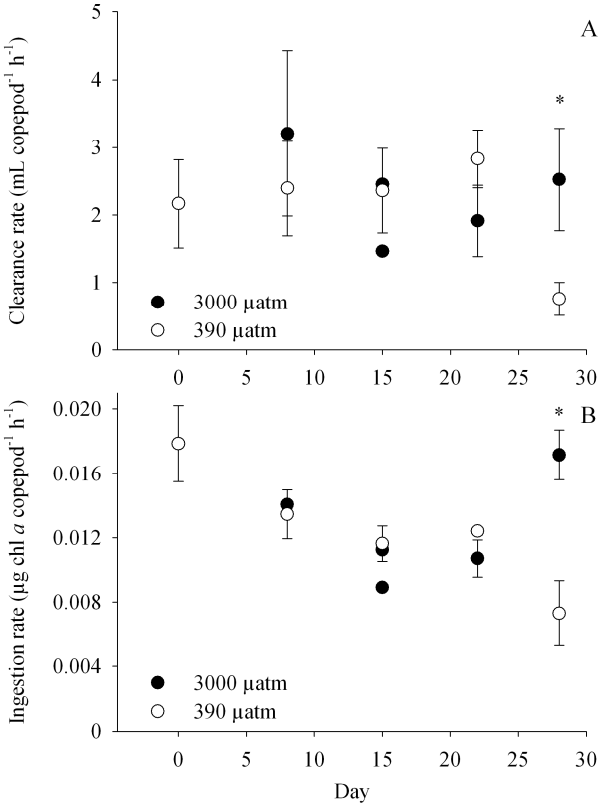

Fig. 1 Long-term response of boreal *C. finmarchicus* to OA: clearance rate (A) and ingestion rate (B) at control (white circles) and high pCO_2 (black circles). Asterisks (*) mark significant differences between CO_2 treatments.

found at day 28, when both clearance and ingestion rates were higher in 3000 µatm as compared to 390 µatm seawater (t-test, CR: $p = 0.018$, IR: $p = 0.003$). Also, sudden changes in pCO_2 did not affect the grazing activity (Fig. 2): In copepods transferred from control to high CO_2 and vice versa, the CR ranged between 0.93 and 3.95 mL copepod^{-1} h^{-1} and the IR between 0.009 and 0.018 µg chlorophyll *a* copepod^{-1} h^{-1}. Significant differences were found only in CR at day 15 (t-test: $p = 0.016$) when

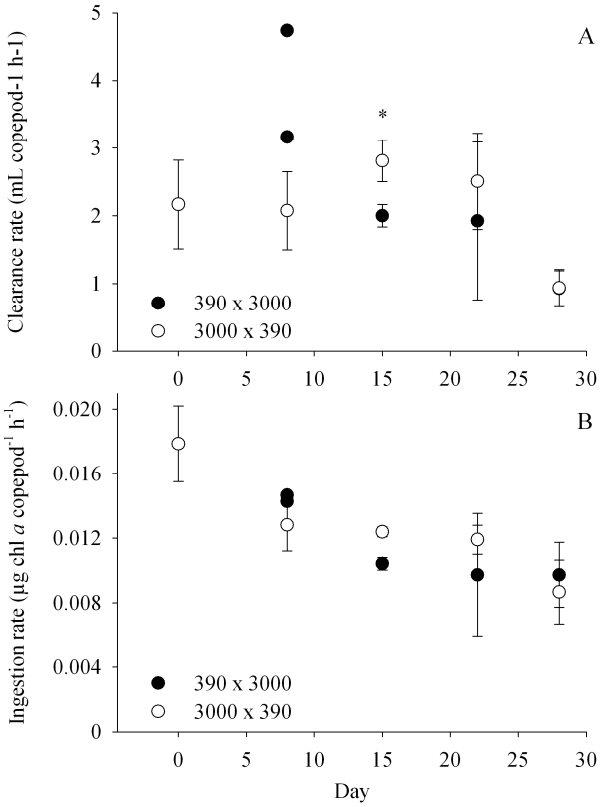

Fig. 2. Immediate response of boreal *C. finmarchicus* to OA: clearance rate (A) and ingestion rate (B) of copepods originating from the high CO_2 mesocosm that were incubated at control pCO_2 (white circles) and of copepods originating from the control mesocosm that were incubated at high pCO_2 (black circles). Asterisks (*) mark significant differences between CO_2 treatments.

copepods transferred from high to control CO_2 had higher CR than the copepods transferred from control to high CO_2.

The mean C and N contents of *C. finmarchicus* CV (2.0 ± 0.2 mm prosome length) from the control mesocosms were 161 and 19 µg, respectively (Table 3), and did not change significantly over time (Table 4). In copepods from the high CO_2 mesocosm, C and N contents were significantly lower as compared to copepods from control mesocosms on day 8 and 15 (t-test, $0.001 \leq p \leq 0.008$). On the last two sampling days, however, C and N had increased, and no differences in body mass were found between copepods from high and control mesocosms (Table 3, 4).

In Arctic *C. finmarchicus* CV sampled in Fram Strait, grazing rates were lower as compared to those measured in the boreal population from Raunefjord. Their CR varied

Table 3 Carbon and nitrogen contents of *Calanus finmarchicus* CV sampled from control and high CO_2 mesocosms.

	Control CO_2			High CO_2		
day	**C (µg)**	**N (µg)**	**n**	**C (µg)**	**N (µg)**	**n**
0	151 ± 34	19 ± 2	19	151 ± 34	19 ± 2	19
8	167 ± 26	19 ± 2	11	134 ± 17	17 ± 1	8
15	167 ± 26	19 ± 2	12	123 ± 23	16 ± 2	12
22	162 ± 12	19 ± 1	12	158 ± 12	18 ± 1	12
28	164 ± 25	18 ± 2	12	169 ± 26	19 ± 2	12

Values are presented as mean ± standard deviation. C: carbon content, N: nitrogen content, n: number of samples. For each sample, 2 - 6 copepods (usually 5) were pooled.

Table 4 Spearman rank order correlation analyses for the development of carbon and nitrogen content in the *Calanus* spp. throughout the CO_2 experiments.

Species	**Population**	**[CO_2] (µatm)**	**Para-meter**	**Correlation coefficient**	**p**
C. finmarchicus	Boreal	390	C	0.167	0.180
C. finmarchicus	Boreal	3000	C	0.256	**0.043**
C. finmarchicus	Boreal	390	N	0.019	0.881
C. finmarchicus	Boreal	3000	N	0.139	0.275
C. finmarchicus	Arctic	390	C	-0.254	**0.004**
C. finmarchicus	Arctic	1120	C	-0.174	0.052
C. finmarchicus	Arctic	3000	C	-0.318	**<0.001**
C. finmarchicus	Arctic	390	N	-0.027	0.760
C. finmarchicus	Arctic	1120	N	0.052	0.565
C. finmarchicus	Arctic	3000	N	-0.169	0.062
C. glacialis	Arctic	390	C	0.117	0.200
C. glacialis	Arctic	1120	C	0.391	**<0.001**
C. glacialis	Arctic	3000	C	0.299	**<0.001**
C. glacialis	Arctic	390	N	0.471	**<0.001**
C. glacialis	Arctic	1120	N	0.625	**<0.001**
C. glacialis	Arctic	3000	N	0.596	**<0.001**

[CO_2]: CO_2 concentration; C: carbon content; N: nitrogen content. Values presented in **bold** indicate significant correlations between C and N content and time.

from 0 to 0.57 mL copepod^{-1} h^{-1} (Fig. 3A), the IR ranged between 0 and 0.003 µg chlorophyll *a* copepod^{-1} h^{-1} (Fig. 3B). Again, CO_2 did not affect the grazing activity. The body mass of the Arctic *C. finmarchicus* (2.6 ± 0.1 mm) was almost twice as high as that of the boreal population (Table 5). Corresponding to the low ingestion rates, the C content, decreased significantly from 329 µg to less than 300 µg copepod^{-1} in the control and the high CO_2 treatment over time (Table 4); at 1120 µatm CO_2 it also declined but not statistically significant ($p = 0.052$). The N content was 32 µg copepod^{-1} at the beginning and did not change significantly during the experiment. CO_2 did in general not affect the body mass of the copepods. The only exception was day 12 (one-way ANOVA, $p \leq 0.019$) when both C and N content were significantly higher in CV

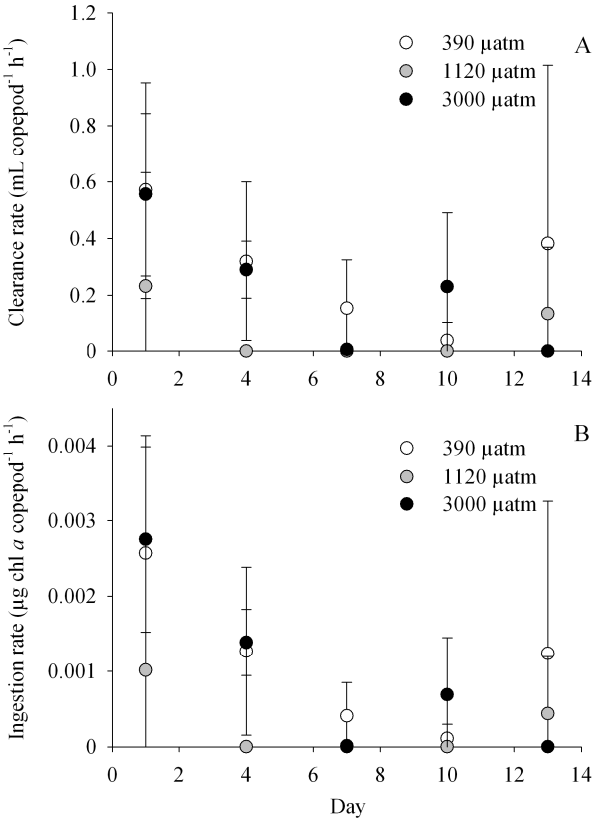

Fig. 3. Response of Arctic *C. finmarchicus* to OA: clearance rate (A) and ingestion rate (B) at control (white circles), intermediate (grey circles) and high pCO_2 (black circles).

incubated at 1120 µatm as compared to 3000 µatm CO_2 (post-hoc Holm-Sidak test, C: $p = 0.007$; N: $p = 0.001$).

In *C. glacialis* CV (Fig. 4), mean clearance rates were 1.44 to 4.67 mL copepod^{-1} h^{-1}, and ingestion rates varied from 0.003 to 0.007 µg chlorophyll *a* copepod^{-1} h^{-1}. Again, there were no significant differences between the treatments, with two exceptions. On day 4, copepods kept at 1120 µatm ingested significantly more chlorophyll *a* than copepods from 390 µatm CO_2 (Tukey test, $p < 0.05$). On day 8, ingestion rates in *C. glacialis* kept at 390 µatm were lower than in the other two treatments (Holm-Sidak test, $p \leq 0.001$).

Table 5 Prosome length, carbon and nitrogen contents of *Calanus* spp. incubated at different CO_2 concentrations.

	C. finmarchicus CV											
	390 µatm CO2				1120 µatm CO2				3000 µatm CO2			
day	Length (mm)	C (µg)	N (µg)	n	Length (mm)	C (µg)	N (µg)	n	Length (mm)	C (µg)	N (µg)	n
0	2.6 ± 0.1	329 ± 77	32 ± 8	36	2.6 ± 0.1	329 ± 77	32 ± 8	36	2.6 ± 0.1	329 ± 77	32 ± 8	36
1		308 ± 36	32 ± 4	6		318 ± 44	34 ± 4	6		310 ± 24	34 ± 2	6
3	2.6 ± 0.2	325 ± 67	33 ± 7	12	2.6 ± 0.2	305 ± 81	31 ± 7	12	2.5 ± 0.2	301 ± 76	30 ± 6	11
4		292 ± 19	31 ± 3	6		326 ± 19	35 ± 2	6		306 ± 72	32 ± 9	3
6	2.5 ± 0.2	279 ± 64	29 ± 5	7	2.6 ± 0.1	297 ± 83	32 ± 5	7	2.5 ± 0.2	270 ± 82	29 ± 8	7
7		326 ± 22	35 ± 2	4		308 ± 15	33 ± 1	3		308 ± 29	33 ± 3	4
9	2.6 ± 0.1	292 ± 64	31 ± 6	8	2.7 ± 0.1	330 ± 58	36 ± 5	7	2.6 ± 0.1	335 ± 54	35 ± 6	7
10		318 ± 34	34 ± 3	6		295 ± 31	32 ± 4	6		299 ± 35	32 ± 3	6
12	2.6 ± 0.2	297 ± 67	32 ± 6	37	2.6 ± 0.1	324 ± 41	35 ± 4	36	2.6 ± 0.1	288 ± 52	31 ± 5	36
13		297 ± 28	33 ± 2	6		285 ± 30	31 ± 4	6		298 ± 30	32 ± 3	6

	C. glacialis CV											
	390 µatm CO2				1120 µatm CO2				3000 µatm CO2			
day	Length (mm)	C (µg)	N (µg)	n	Length (mm)	C (µg)	N (µg)	n	Length (mm)	C (µg)	N (µg)	n
0	3.5 ± 0.1	646 ± 168	56 ± 12	36	3.5 ± 0.1	646 ± 168	56 ± 12	36	3.5 ± 0.1	646 ± 168	56 ± 12	36
1		789 ± 124	69 ± 9	9		674 ± 105	63 ± 7	9		687 ± 55	63 ± 4	10
4		704 ± 137	64 ± 9	6		743 ± 134	71 ± 11	6		629 ± 94	61 ± 8	9
8		713 ± 74	70 ± 5	8		736 ± 63	71 ± 6	6		702 ± 108	62 ± 22	9
12		719 ± 93	72 ± 9	9		747 ± 60	66 ± 22	9		672 ± 104	65 ± 8	8
15	3.5 ± 0.1	693 ± 159	71 ± 18	44	3.6 ± 0.2	788 ± 140	77 ± 17	41	3.6 ± 0.1	783 ± 132	76 ± 16	43
16		725 ± 85	73 ± 7	9		778 ± 58	78 ± 7	9		685 ± 67	72 ± 8	9

Values are presented as mean ± standard deviation. C: carbon content. N: nitrogen content. n: number of samples; usually 2-6 copepods were pooled for analyses (n highlighted in **bold**); single copepods measurements are indicated by regular numbers.

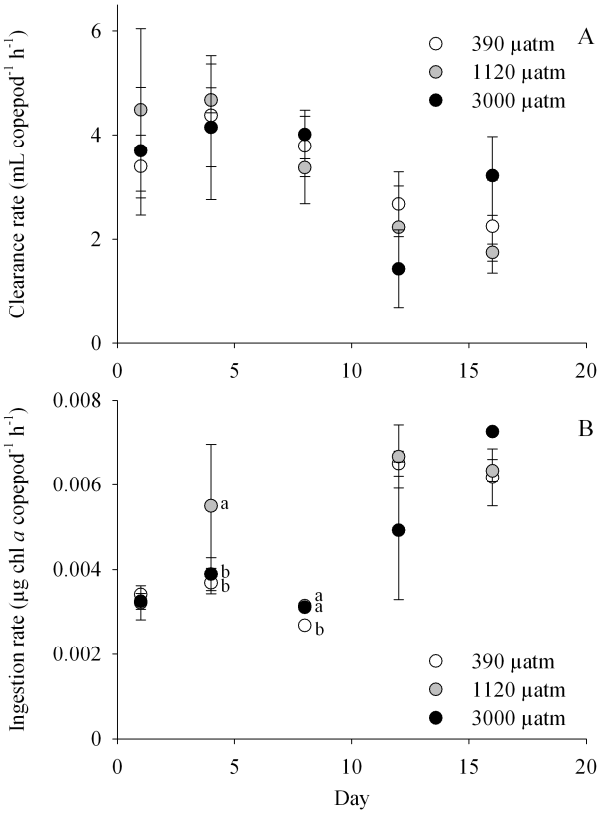

Fig. 4. Response of *C. glacialis* to OA: clearance rate (A) and ingestion rate (B) at control (white circles), intermediate (grey circles) and high pCO_2 (black circles). Significant differences among CO_2 treatments are marked by different lower case letters.

The C and N contents of *C. glacialis* (3.5 ± 0.1 mm) at the start of the incubation were 646 µg and 56 µg, respectively (Table 5). Over time, the N content increased significantly by 30 to 40 % at all CO_2 concentrations. The carbon content increased significantly only in copepods kept at 1120 and 3000 µatm CO_2 (Table 6). The pCO_2 did not have significant effects on body mass, with two exceptions. On day 16, the C content in copepods from 390 µatm was significantly lower as compared to the other CO_2 treatments (Holm-Sidak test, $p \leq 0.004$), whereas on day 17 copepods from 3000 µatm had significantly more body carbon than those from 1120 µatm CO_2 ($p = 0.011$).

4 DISCUSSION

The uptake of CO_2 by the world's oceans and the resulting surface ocean acidification (OA) have the potential to either increase or decrease feeding rates of marine organisms. In the common sea star *Asterias rubens*, for example, which does not compensate for

elevated extracellular pCO_2 under environmental hypercapnia, feeding rates are lower when exposed to OA than under normocapnic conditions (Appelhans et al. 2012). Also in larval sea urchins, i.e. *Strongylocentrotus droebachiensis* and *Dendraster excentricus*, and in larvae of the gastropod *Concholepas concholepas*, food uptake was significantly impaired by elevated seawater pCO_2 (Dupont and Thorndike 2008, Chan et al. 2011, Vargas 2013). Other species regulate the extracellular pH such as the shore crab *Carcinus maenas*. Feeding in this species was reduced at high as compared to low pCO_2 during a 10-week incubation experiment, but not in short-term feeding assays (Appelhans et al. 2012). The authors suggested that during long-term exposure additional energy had to be allocated to acid-base regulation, which affected energy demanding process related to feeding such as digestion or prey handling (Appelhans et al. 2012). In contrast, the Antarctic krill *Euphausia superba* ingested more food at elevated pCO_2 (Saba et al. 2012), and this study discussed that the increase in feeding compensated for elevated energetic needs due to acid-base regulation. Li and Gao (2012) came to a similar conclusion in their study on the copepod *Centropages typicus*, however, the differences in grazing rates were not significant.

The Arctic populations of *C. glacialis* and *C. finmarchicus* were incubated at relatively stable CO_2 concentrations throughout our experiments, simulating a realistic scenario that is projected to occur in about the year 2100 (1120 µatm) and an extreme scenario (3000 µatm) that is not realistic for surface waters (Caldeira and Wickett 2003). The boreal *C. finmarchicus* were sampled from large-scale mesocosms, which were initially adjusted to 390 and 3000 µatm CO_2. In the high CO_2 mesocosm, the pH increased over time by 0.2 - 0.6 units, depending on the water depth and thus, *C. finmarchicus* in this mesocosm were not kept at a stable pCO_2. There were, however, marked differences in pH between control and high CO_2 mesocosms throughout the study, which allowed for detecting possible changes in feeding activity due to OA.

Our study indicates that elevated seawater pCO_2 did not directly affect the grazing activity of *C. finmarchicus* and *C. glacialis*, neither after sudden changes in pCO_2 nor during two to four weeks of exposure. We found significant differences in grazing between copepods from control and high pCO_2 treatments only on few days and there was no general trend to increased or decreased grazing activity under OA scenarios. All copepods from control and high pCO_2 treatments were fed with diatoms, i.e. *Thalassiosira weissflogii*, which were grown at control conditions, and thus the

copepods received food organisms of the same quality. De Kluijver et al. (2013), in contrast, inferred from calculation of the amount of incorporated ^{13}C during a mesocosm study in an Arctic fjord that the food uptake of *Calanus* spp., decreased at elevated CO_2 concentrations. Their approach, however, addressed both direct and indirect effects of OA, as CO_2 affected the algal community composition in the mesocosms (Leu et al. 2013, Schulz et al. 2013, Brussaard et al. 2013) and therefore the food quality for the copepods.

The grazing rates of *C. finmarchicus* from the Fram Strait were extremely low during our experiments on-board RV *Polarstern*, which is in agreement with previous data from this area at the same time of the year (Smith 1988). As *C. finmarchicus* descends to deep waters in July or August in Arctic waters (e.g. Kosobokova 1999, Madsen et al. 2001, Søreide et al. 2010), we believe that such low feeding activities despite high food supply is a sign for transition into the overwintering state. In agreement with the low ingestion rates, the body mass of *C. finmarchius* decreased by up to 1 % of the body C day^{-1}, which was similar to CV kept without food (Mayzaud 1976). *C. glacialis*, in contrast, ingested sufficient material to increase in both body carbon and nitrogen content during the 16 days of incubation.

When ingestion rates are similar, differences in body weight may serve as another indicator for changes in energy demands due to elevated pCO_2, i.e. the copepod body mass may increase to a lesser extend or even decrease if coping with OA stress is energetically costly. In our laboratory experiments, however, the body mass of the copepods from the different CO_2 treatments was not significantly different and, thus, there was no indication for additional energy demands. Only during the mesocosm experiments, we found significantly lower body mass in *C. finmarchicus* CV from the high pCO_2 mesocosm on two days (day 8 and 15). This may be attributed to lower food quality in high pCO_2 mesocosms as the phytoplankton community changed with pCO_2 (J. R. Bermúdez Monsalve (GEOMAR), pers. comm.). At day 14, nutrients were added, which induced a phytoplankton bloom (Schulz and Riebesell, unpubl. data). The food availability thus improved in all mesocosms and this could explain why there were no differences in body mass at later sampling dates.

In conclusion, we did not find any direct effects of elevated pCO_2 on the copepods grazing rates and body mass, neither in *C. finmarchicus* nor in *C. glacialis* and this suggests that the energy demand of these two *Calanus* species does not increase at OA. The mechanisms responsible for the tolerance to OA in *C. finmarchicus* and *C. glacialis*, as was also reported in other studies (Mayor et al. 2007, Weydmann et al. 2012, Hildebrandt et al. in press), remain unknown. Recent studies on Antarctic copepods revealed that species, which overwinter in a diapause similar to the Arctic *Calanus* spp., strongly regulate their extracellular acid-base status during winter, exhibiting pH values as low as 5.7 (Sartoris et al. 2010, Schründer et al. 2013). Compensating for comparably small shifts in seawater pH due to environmental hypercapnia might therefore not be a challenge for these copepods.

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REFERENCES

Alcamo, J., Bouwman, A., Edmonds, J., Grübler, A., Morita, T. and Sugandhy, A. (1995) An evaluation of the IPCC IS92 emission scenarios. In: Houghton, J. T., Meira Filho, L. G., Bruce, J., Lee, H., Callander, B. A., Haites, E., Harris, N. and Maskell, K. (eds.) *Climate change 1994*. Cambridge University Press, Cambridge, New York, Melbourne, pp. 247-304.

Appelhans, Y. S., Thomsen, J., Pansch, C., Melzner, F. and Wahl, M. (2012) Sour times: seawater acidification effects on growth, feeding behaviour and acid-base status of *Asterias rubens* and *Carcinus maenas*. *Mar. Ecol. Prog. Ser.* **459**, 85-97.

Bartram, W. C. (1980) Experimental development of a model for the feeding of neritic copepods on phytoplankton. *J. Plankton Res.* **3** (1), 25-51.

Brussaard, C. P. D., Noordeloos, A. A. M., Witte, H., Collenteur, M. C. J., Schulz, K., Ludwig, A. and Riebesell, U. (2013) Arctic microbial community dynamics influenced by elevated CO_2 levels. *Biogeosciences* **10**, 719-731.

Caldeira, K. and Wickett, M. E. (2003) Anthropogenic carbon and ocean pH. *Nature* **425**, 365.

Chan, K. Y. K., Grünbaum, D. and O'Donnell, M. J. (2011) Effects of ocean-acidification-induced morphological changes on larval swimming and feeding. *J. Exp. Biol*. **214**, 3857-3867.

Conover, R. J. (1988) Comparative life histories in the genera *Calanus* and *Neocalanus* in high latitudes of the northern hemisphere. *Hydrobiologia* **167/168**, 127-142.

De Kluijver, A., Soetaert, K., Czerny, J., Schulz, K. G., Boxhammer, T., Riebesell, U. and Middelburg, J. J. (2013) A ^{13}C labeling study on carbon fluxes in Arctic plankton communities under elevated CO_2 levels. *Biogeosciences* **10**, 1425-1440.

Dupont, S. and Thorndyke, M. C. (2008) Ocean acidification and its impact on the early life-history stages of marine animals. *CIESM Workshop Monographs* **36**, 89-97.

Falk-Petersen, S., Pavlov, V., Timofeev, S. and Sargent, J. R. (2007) Climate variability and possible effects on arctic food chains: The role of *Calanus*. In: Ørbæk, J. B., Kallenborn, R., Tombre, I., Hegseth, E. N., Falk-Petersen, S. and Hoel, A. H. (eds.) *Arctic alpine ecosystems and people in a changing environment*. Springer, Berlin, Heidelberg, pp. 147-166.

Friedlingstein, P., Houghton, R. A., Marland, G., Hackler, J., Boden, T. A., Conway, T. J., Canadell, J. G., Raupach, M. R., Ciais, P. and Le Quéré, C. (2010) Update on CO_2 emissions. *Nature Geoscience* **3**, 811-812.

Frost, B. W. (1972) Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. *Limnol. Oceanogr.* **17** (6), 805-815.

Gentsch, E., Kreibich, T., Hagen, W. and Niehoff, B. (2009) Dietary shifts in the copepod *Temora longicornis* during spring: evidence from stable isotope signatures, fatty acid biomarkers and feeding experiments. *J. Plankton Res*. **31** (1), 45-60.

Guillard, R. R. L. (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith, W. L. and Chanley, M. H. (eds.) *Culture of Marine Invertebrate Animals*. Plenum Press, New York, pp. 26-60.

Hildebrandt, N., Niehoff, B. and Sartoris, F. J. (in press) Long-term effects of elevated CO_2 and temperature on the Arctic calanoid copepods *Calanus glacialis* and *C. hyperboreus*. *Mar. Pollut. Bull.*

Hirche, H.-J. (1998) Dormancy in three *Calanus* species (*C. finmarchicus*, *C. glacialis* and *C. hyperboreus*) from the North Atlantic. *Arch. Hydrobiol. Spec. Issues Advanc. Limnol*. **52**, 359-369.

Jaschnov, W. A. (1970) Distribution of *Calanus* species in the seas of the northern hemisphere. *Int. Revue ges. Hydrobiol*. **55** (2), 197-212.

Kosobokova, K. N. (1999) The reproductive cycle and life history of the Arctic copepod *Calanus glacialis* in the White Sea. *Polar Biol*. **22**, 254-263.

Kurihara, H., Shimode, S. and Shirayama, Y. (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.

Lee, R. F. (1974) Lipid composition of the copepod *Calanus hyperboreas* from the Arctic ocean. Changes with depth and season. *Mar. Biol*. **26**, 313-318.

Leu, E., Daase, M., Schulz, K. G., Stuhr, A. and Riebesell, U. (2013) Effect of ocean acidification on the fatty acid composition of a natural plankton community. *Biogeosciences* **10**, 1143-1153.

Li, W. and Gao, K. (2012) A marine secondary producer respires and feeds more in a high CO_2 ocean. *Mar. Pollut. Bull.* **64**, 699-703.

Longhurst, A. R. (1985) The structure and evolution of plankton communities. *Prog. Oceanogr.* **15**, 1-35.

Madsen, S. D., Nielsen, T. G. and Hansen, B. W. (2001) Annual population development and production by *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* in Disko Bay, western Greenland. *Mar. Biol*. **139**, 75-93.

Marshall, S. M. and Orr, A. P. (1955) On the biology of *Calanus finmarchicus* VIII. Food uptake, assimilation and excretion in adult and stage V *Calanus*. *J. Mar. Biol. Ass. U.K*. **34**, 495-529.

Mayor, D. J., Matthews, C., Cook, K., Zuur, A. F. and Hay, S. (2007) CO_2-induced acidification affects hatching success in *Calanus finmarchicus*. *Mar. Ecol. Prog. Ser.* **350**, 91-97.

Mayor, D. J., Everett, N. R. and Cook, K. B. (2012) End of century ocean warming and acidification effects on reproductive success in a temperate marine copepod. *J. Plankton Res*. **34** (3), 258-262.

Mayzaud, P. (1976) Respiration and nitrogen excretion of zooplankton. IV. The influence of starvation on the metabolism and the biochemical composition of some species. *Mar. Biol*. **37**, 47-58.

McConville, K., Halsband, C., Fileman, E. S., Somerfield, P. J. and Findlay, H. S. (2013) Effects of elevated CO_2 on the reproduction of two calanoid copepods. *Mar. Pollut. Bull.* http://dx.doi.org/10.1016/j.marpolbul.2013.02.010.

Melzner, F., Stange, P., Trübenbach, K., Thomsen, J., Casties, I., Panknin, U., Gorb, S. N. and Gutowska, M. A. (2011) Food supply and seawater pCO_2 impact calcification and internal shell dissolution in the blue mussel *Mytilus edulis*. *PLoS ONE* **6** (9), e24223. doi:10.1371/journal.pone.0024223.

Michaelidis, B., Ouzounis, C., Paleras, A. and Pörtner, H. O. (2005) Effects of long-term moderate hypercapnia on acid-base balance and growth rate in marine mussels *Mytilus galloprovincialis*. *Mar. Ecol. Prog. Ser.* **293**, 109-118.

Miles, H., Widdicombe, S., Spicer, J. I. and Hall-Spencer, J. (2007) Effects of anthropogenic seawater acidification on acid-base balance in the sea urchin *Psammechinus miliaris*. *Mar. Pollut. Bull.* **54**, 89-96.

Niehoff, B., Schmithüsen, T., Knüppel, N., Daase, M., Czerny, J. and Boxhammer, T. (2013) Mesozooplankton community development at elevated CO_2 concentrations: results from a mesocosm experiment in an Arctic fjord. *Biogeosciences* **10**, 1391-1406.

Pasternak, A., Arashkevich, E., Tande, K. and Falkenhaug, T. (2001) Seasonal changes in feeding, gonad development and lipid stores in *Calanus finmarchicus* and *C. hyperboreus* from Malangen, northern Norway. *Mar. Biol*. **138**, 1141-1152.

Pedersen, S. A., Hansen, B. H., Altin, D. and Olsen, A. J. (2013) Chronic exposure of the North Atlantic copepod *Calanus finmarchicus* (Gunnerus, 1770) to CO_2-acidified seawater; effects on survival, growth and development. *Biogeosciences Discuss*. **10**, 5273-5300.

Pörtner, H. O., Reipschläger, A. and Heisler, N. (1998) Acid-base regulation, metabolism and energetics in *Sipunculus nudus* as a function of ambient carbon dioxide level. *J. Exp. Biol*. **201**, 43-55.

Raven, J., Caldeira, K., Elderfield, H., Hoegh-Guldberg, O., Liss, P., Riebesell, U., Shepherd, J., Turley, C. and Watson, A. (2005) Ocean acidification due to increasing atmospheric carbon dioxide. Policy document 12/05, The Royal Society, UK.

Reipschläger, A. and Pörtner, H. O. (1996) Metabolic depression during environmental stress: The role of extracellular versus intracellular pH in *Sipunculus nudus*. *The Journal of Exp. Biol.* **199**, 1801-1807.

Riebesell, U., Czerny, J., von Bröckel, K., Boxhammer, T., Büdenbender, J., Deckelnick, M., Fischer, M., Hoffmann, D., Krug, S. A., Lentz, U., Ludwig, A., Muche, R. and Schulz, K. G. (2013) Technical note: A mobile sea-going mesocosm system - new opportunities for ocean change research. *Biogeosciences* **10**, 1835-1847.

Roman, M. R. and Rublee, P. A. (1980) Containment effects in copepod grazing experiments: A plea to end the black box approach. *Limnol. Oceanogr.* **25** (6), 982-990.

Rosa, R. and Seibel, B. A. (2008) Synergistic effects of climate-related variables suggest future physiological impairment in a top oceanic predator. *PNAS* **105** (52), 20776-20780.

Saba, G. K., Schofield, O., Torres, J. J., Ombres, E. H. and Steinberg, D. K. (2012) Increased feeding and nutrient excretion of adult Antarctic krill, *Euphausia superba*, exposed to enhanced carbon dioxide (CO_2). *PLoS ONE* **7** (12), e52224. doi:10.1371/journal.pone.0052224.

Sartoris, F. J., Thomas, D. N., Cornils, A. and Schnack-Schiel, S. B. (2010) Buoyancy and diapause in Antarctic copepods: The role of ammonium accumulation. *Limnol. Oceanogr*. **55** (5), 1860-1864.

Schründer, S., Schnack-Schiel, S. B., Auel, H. and Sartoris, F. J. (2013) Control of diapause by acidic pH and ammonium accumulation in the hemolymph of Antarctic copepods. *PLoS ONE* **8** (10), e77498. doi:10.1371/journal.pone.0077498.

Schulz, K. G., Bellerby, R. G. J., Brussaard, C. P. D., Büdenbender, J., Czerny, J., Engel, A., Fischer, M., Koch-Klavsen, S., Krug, S. A., Lischka, S., Ludwig, A., Meyerhöfer, M., Nondal, G., Silyakova, A., Stuhr, A. and Riebesell, U. (2013) Temporal biomass dynamics of an Arctic plankton bloom in response to increasing levels of atmospheric carbon dioxide. *Biogeosciences* **10**, 161-180.

Seibel, B. A., Maas, A. E. and Dierssen, H. M. (2012) Energetic plasticity underlies a variable response to ocean acidification in the pteropod, *Limacina helicina antarctica*. *PLoS ONE* **7** (4), e30464. doi:10.1371/journal.pone.0030464.

Smith, S. L. (1988) Copepods in Fram Strait in summer: Distribution, feeding and metabolism. *J. Mar. Sci*. **46**, 145-181.

Solomon, S., Quin, D., Manning, M., Alley, R. B., Berntsen, T., Bindoff, N. L., Chen, Z., Chidthaisong, A., Gregory, J. M., Hegerl, G. C., Heimann, M., Hewitson, B., Hoskins, B. J., Joos, F., Jouzel, J., Kattsov, V., Lohmann, U., Matsuno, T., Molina, M., Nicholls, N., Overpeck, J., Raga, G., Ramaswamy, V., Ren, J., Rusticucci, M., Somerville, R., Stocker, T. F., Whetton, P., Wood, R. A. and Wratt, D. (2007) Technical Summary. In: Solomon, S., Quin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M. and Miller, H. L. (eds.) *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge and New York, pp. 20-91.

Søreide, J. E., Leu, E., Berge, J., Graeve, M. and Falk-Petersen, S. (2010) Timing of blooms, algal food quality and *Calanus glacialis* reproduction and growth in a changing Arctic. *Global Chang. Biol*. **16**, 3154-3163.

Tande, K. S., Hassel, A. and Slagstad, D. (1985) Gonad maturation and possible life cycle strategies in *Calanus finmarchicus* and *Calanus glacialis* in the northwestern part of the Barents Sea. In: Gray, J. S. and Christiansen, M. E. (eds.) *Marine Biology of Polar Regions and Effects of Stress on Marine Organisms*. J. Wiley & Sons Ltd, New York, pp. 141-155.

Tans, P. (2013) Trends in Atmospheric Carbon Dioxide. NOAA/ESRL, http://www.esrl.noaa.gov/gmd/ccgg/trends/.

Thomsen, J. and Melzner, F. (2010) Moderate seawater acidification does not elicit long-term metabolic depression in the blue mussel *Mytilus edulis*. *Mar. Biol.* **157**, 2667-2676.

Vargas, C. A., de la Hoz, M., Aguilera, V., San Martin, V., Manríquez, P. H., Navarro, J. M., Torres, R., Lardies, M. A. and Lagos, N. A. (2013) CO_2-driven ocean acidification reduces larval feeding efficiency and changes food selectivity in the mollusk *Concholepas concholepas*. *J. Plankton Res*. **35** (5), 1059-1068.

Weydmann, A., Søreide, J. E., Kwasniewski, S. and Widdicombe, S. (2012) Influence of CO_2-induced acidification on the reproduction of a key Arctic copepod *Calanus glacialis*. *J. Exp. Mar. Biol. Ecol.* **428**, 39-42.

Widdicombe, S. and Spicer, J. I. (2008) Predicting the impact of ocean acidification on benthic biodiversity: What can animal physiology tell us? *J. Exp. Mar. Biol. Ecol.* **366***,* 187-197.

Wood, H. L., Spicer, J. I. and Widdicombe, S. (2008) Ocean acidification may increase calcification rates, but at a cost. *Proc. R. Soc. B* **275**, 1767-1773.

Zhang, D., Li, S., Wang, G. and Guo, D. (2011) Impacts of CO_2-driven seawater acidification on survival, egg production rate and hatching success of four marine copepods. *Acta Oceanol. Sin.* **30** (6), 86-94.

PUBLICATION III

Ocean acidification effects on a boreal mesozooplankton community - a mesocosm study

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ABSTRACT

Laboratory studies have shown that ocean acidification (OA) can severely impair the performance of marine zooplankton. Not much is known, however, on how plankton communities respond to OA under near-natural conditions. Within the framework of the SOPRAN (Surface Ocean Processes in the Anthropocene) mesocosm study 2011 in Bergen, Norway, our study analyses whether abundance and taxonomic composition of a boreal mesozooplankton community change at elevated seawater pCO_2. Nine large-scale mesocosms (~75 m^3), which were deployed in the Raunefjord in May/June 2011 and adjusted to CO_2 levels ranging from 280 to 3000 µatm, were sampled weekly for ~1 month with an Apstein net (55 µm mesh size). In addition, samples from the sediment traps of the mesocosms, which collected dead and vertically migrating organisms, were taken daily. A taxonomic analysis of water and sediment trap samples showed that the mesozooplankton community was dominated by copepods (*Calanus finmarchicus*, *Pseudocalanus elongatus*, *Temora longicornis*, *Oithona similis*). Other groups occurring regularly were meroplanktonic larvae (bivalves, gastropods, bryozoans, cirripeds, polychaetes, echinoderms), appendicularians, cnidarians and cladocerans. Abundance of *P. elongatus* nauplii as well as bivalve and gastropod larvae was negatively correlated to seawater pCO_2. In the other zooplankton groups, no CO_2 related effects were found. OA induced changes in abundance were not strong enough to alter the community diversity in the water column of the mesocosms, however, in the sediment traps, significant correlations between diversity and CO_2 were evident. Thus, OA might significantly affect the zooplankton community structure in the long term.

KEYWORDS: Carbon dioxide • Community development • Zooplankton abundance • Climate change • Raunefjord • Mesocosms

1 INTRODUCTION

The continuous uptake of man-made carbon dioxide (CO_2) by the world's oceans leads to severe changes in seawater chemistry, i.e. decreases in pH, carbonate ion concentration and calcite and aragonite saturation states (e.g. Caldeira and Wickett 2003; Orr et al. 2005, Feely et al. 2009). This process of ocean acidification (OA) has been shown to affect a number of marine animals. Despite some intraspecific variability, calcifying species such as corals, molluscs and echinoderms are probably among the most threatened organisms as their ability to build and maintain their skeletal structures can be severely hampered (e.g. Ries et al. 2009, Melzner et al. 2011, Comeau et al. 2012). Other processes can, however, also be influenced by elevated seawater pCO_2, such as survival of juveniles and adults (e.g. Dupont et al. 2008, Kurihara et al. 2008, Zhang et al. 2011, Gonzales-Bernat et al. 2013), metabolic rates (e.g. Michaelidis et al. 2005, Rosa and Seibel 2008, Li and Gao 2012, Saba et al. 2012), growth (e.g. Dupont and Thorndyke 2008, Kurihara et al. 2008, Fitzer et al. 2012), reproduction (e.g. Havenhand et al. 2008, Kawaguchi et al. 2011, Zhang et al. 2011) and behaviour (Ellis et al. 2009, Munday et al. 2009, 2010). Thus, non-calcifying organisms can also be negatively affected by OA.

Many studies up to date focussed on direct effects of an acidified environment on single species. Such laboratory studies are appropriate to tackle changes in physiological and ecological processes within an organism, but they do not take into account indirect effects of OA, e.g. species interactions or altered food web structures. Urabe et al. (2003) and Rossoll et al. (2012) have shown in two-species experiments, for example, that elevated pCO_2 can alter the chemical composition of algal species, which in turn affected the growth of the freshwater cladoceran *Daphnia pulicaria* and growth and reproduction in the calanoid copepod *Acartia tonsa*, which fed on these algae. However, the response of algae to OA is species specific, and when feeding on a mixed-species assemblage, the indirect food effects of OA on herbivores might be mitigated (Urabe and Waki 2009, Rossoll et al. 2013).

Mesocosm studies are powerful tools to investigate the impact of increasing CO_2 concentrations on marine communities under near-natural conditions (Riebesell et al. 2010, Widdicombe et al. 2010). Studies on pelagic community dynamics, mainly of bacteria, phyto- and microzooplankton, in response to OA under near-natural conditions

have up to date been conducted in mesocosm facilities at Espregrend, the Marine Biological Station of the University of Bergen, Norway and at Jangmok, South Korea as well as in offshore mesocosms in the Arctic Kongsfjord in Svalbard (e.g. Engel et al. 2004, 2008, Delille et al. 2005, Grossart et al. 2006, Kim et al. 2006, 2011, Allgaier et al. 2008, Suffrian et al. 2008, Brussaard et al. 2013). The development of the mesozooplankton community at elevated CO_2 concentrations was investigated in two studies. The community in the Kongsfjord, which was dominated by meroplanktonic larvae, did not change significantly during 30 days at CO_2 concentrations that initially ranged from 185 to 1420 µatm (Niehoff et al. 2013). However, as the time span of this study might not have been sufficient to detect changes in slowly developing Arctic communities, Niehoff et al. (2013) pointed out that studies at lower latitudes, where species generally have shorter life cycles, are needed. Troedsson et al. (2012) investigated the effects of OA on a plankton community in a 15 day, small-scale (2.5 m^3) mesocosm experiment in Bergen, Norway. Here, the abundance of appendicularians significantly increased at a pCO_2 of 1000 µatm as compared to control conditions (380 µatm). The other zooplankton taxa, mainly polychaetes, rotifers and copepods, were unaffected, suggesting that appendicularians might occupy an increasingly important role in future oceans (Troedsson et al. 2012).

The present study was part of the SOPRAN (Surface Ocean Processes in the Anthropocene) 2011 mesocosm experiment in May and June 2011, which was conducted at Espegrend to evaluate the effects of increasing seawater pCO_2 on a natural pelagic plankton community and the biogeochemical cycling. Nine large Kiel Off-Shore Mesocosms for future Ocean Simulation (KOSMOS; ~75 m^3) containing natural pelagic communities were adjusted to eight different CO_2 concentrations between 280 to 3000 µatm, thereby covering the range of pCO_2 levels that are included in the RCP emissions scenarios for the next three centuries (Moss 2010, Meinshausen et al. 2011) as well as an extreme scenario, and run for 35 days. Within this framework, our study aimed to investigate whether direct and indirect effects of OA on mesozooplankton species that were reported from laboratory experiments (e.g. Findlay et al. 2009, Comeau et al. 2010, Rossoll et al. 2012, Stumpp et al. 2013) persist under near-natural conditions in a large-scale mesocosm setup, and if so, whether the zooplankton community as a whole will change. For this purpose, we regularly sampled and analysed the mesozooplankton

community in terms of abundance and taxonomic composition in the water column and the sediment traps of the nine mesocosms as well as in the fjord.

2 METHODS

2.1 Mesocosm setup

This study was part of the SOPRAN mesocosm experiment in 2011 at Espegrend, the Marine Biological Station of the University of Bergen, Norway. In May/June 2011, nine KOSMOS mesocosms designed by GEOMAR, Kiel, Germany, were deployed in the Raunefjord at N 60° 15.87', E 005° 12.33' for six weeks. For technical details on the mesocosms and their deployment see Riebesell et al. (2013). The mesocosms in our study were 25 m long and enclosed approx. 75 m^3 of fjord water. During deployment, the openings of the mesocosm bags were covered by a mesh (3 mm mesh size) which prevented larger organisms from entering the mesocosms. On the lower end of each mesocosm bag, a sediment trap was installed to collect material sinking out of the water column. For three days after deployment, the water in the mesocosms was able to exchange with the surrounding fjord water. Then, the mesocosms were closed. The exact volume of the water in the mesocosms was calculated by adding salt solution and measuring the salinity before (31.6 psu) and after the addition (32.0 psu; J. Czerny, GEOMAR, unpubl. data). CTD casts were conducted daily in the mesocosms and in the fjord to monitor temperature and salinity. The temperature increased from approx. 7 to 10 °C until day 15 of the experiment; thereafter it did not change (K. G. Schulz, unpubl. data). Salinity decreased only slightly throughout the experiment, mostly in the upper water column (K. G. Schulz, unpubl. data).

To study the effects of OA on a near-natural plankton community, the pCO_2 in seven of the mesocosms was manipulated by adding different amounts of CO_2 saturated filtrated fjord water equally over the whole water column in five steps on five consecutive days. The day of the first CO_2 addition is defined as the start of the experiment (t_0; 8 May). The CO_2 target levels were 390 (mesocosm M6), 560 (M8), 840 (M1), 1120 (M3), 1400 (M5), 2000 (M7) and 3000 µatm (M9). M2 and M4 served as controls at ~280 µatm CO_2, which was equal to the ambient fjord condition at the time of the experiment. The initial pH values (on a total scale) in the mesocosms after CO_2 manipulation was completed ranged between 8.2 and 7.2. Over the course of the study, pH in the

mesocosms increased due to gas exchange with the atmosphere and biological activity, especially in the high CO_2 mesocosms (K. G. Schulz, unpubl. data). There was, however, still a pH gradient at the end of the experiment.

Concentrations of chlorophyll *a* and inorganic nutrients in the mesocosms were measured daily from integrated water samples (K. G. Schulz, unpubl. data). Compared to the fjord, inorganic nutrient concentrations were higher in the mesocosms at the beginning of the experiment, and subsequently a phytoplankton bloom developed until approx. t_5. By this time, nutrient concentrations were as low as in the fjord. As a major objective of the study was to investigate phytoplankton dynamics, nitrate (approx. 5 µmol L^{-1}) and phosphate (approx. 0.16 µmol L^{-1}) were added to the mesocosms on t_{14}, thus inducing another bloom, which peaked around t_{20} (K. G. Schulz, unpubl. data). Based on the chlorophyll *a* development, the experimental period can be divided into five phases, which include the start of the experiment (phase 0 until t_2), the first phytoplankton bloom (phase I, t_2-t_9), the first period of nutrient limitation (phase II, t_9-t_{14}), the second phytoplankton bloom (phase III, t_{14}-t_{25}) and the nutrient limited period at the end of the experiment (phase IV, t_{25}-t_{34}).

2.2 Mesozooplankton sampling

The mesozooplankton community in each mesocosm and in the fjord was sampled with an Apstein net (mesh size 55 µm, net opening 17 cm). Sampling depth was limited to 23 m to avoid resuspending material from the sediment traps. The first sampling was performed prior to the first CO_2 addition, the second one after the CO_2 manipulation was completed. Thereafter, samples were taken in approx. weekly intervals (t_{12}, t_{19}, t_{25}, t_{33}). Two net hauls were conducted in each mesocosm and in the fjord on each sampling day. The samples were then brought to Espegrend within approx. 1 h. One net haul from each mesocosm and the fjord was preserved in 4 % formalin buffered with hexamethylenetetramine to analyze the mesozooplankton community. The remaining samples were filtrated on combusted, pre-weighted GF/F filters to determine mesozooplankton biomass.

Material collected in the sediment traps was sampled every morning using a vacuum pump. Subsamples of 10 ml from each mesocosm were preserved with 4 % formalin

buffered with hexamethylenetetramine. In order to reduce the sample number, the subsamples of two subsequent days were pooled for each mesocosm.

Both, water and sediment trap samples were analyzed for zooplankton abundance and community composition under a stereomicroscope. Organisms were determined to the lowest taxonomical level, if possible to species and developmental stage (copepods). Sediment trap samples were analyzed completely, and abundances were calculated as total numbers of settled organisms 2 $days^{-1}$. The water samples from the fjord and the mesocosms were splitted with a plankton splitter (HydroBios, Kiel, Germany) into subsamples of 1/8 (1 of 60 samples), 1/16 (53 samples) and 1/32 (6 samples) before the analysis. Abundant species were sorted from only one subsample, whereas rare species were sorted from at least two subsamples. Mesozooplankton abundances were calculated as individuals m^{-3}.

To determine mesozooplankton biomass in the mesocosms and in the fjord, the filters with the mesozooplankton samples were dried at 55 °C for 48 hours and then weighted. Biomass was calculated in terms of dry weight m^{-3}.

2.3 Statistics

To account for changes in the zooplankton community in the water column and in the sediment traps of the mesocosms, linear mixed effects models (LME) of diversity measure H (Shannon-index, see Oksanen et al. 2013) were fitted within lme4 (Bates et al. 2013) using R (version 3.02; R Core Team 2013), a method that was previously applied by Niehoff et al. (2013). We included ten taxa in the analysis, i.e. Copepoda, Bivalvia, Gastropoda, Echinodermata, Appendicularia, Bryzoa, Cirripedia, Cladocera, Cnidaria and Polychaeta, which all occurred regularly in the samples. The repeated measures within mesocosms were incorporated as random effects by the CO_2 concentration as a factor specific to mesocosms. To incorporate the second (induced) phytoplankton bloom (after day 14), the two time domains were distinguished as a factor denoted “Nutr” (before day 14, after day 14). In the fixed effects, the most complex model contained a three-way interaction of Nutr*CO_2*Day. For model selection, removal of three- and two-way terms was applied, dropping non-significant terms ($p > 0.05$, ANOVA). The resulting models are displayed in plots of time courses of H including the modelled response as straight lines.

Spearman rank tests were performed using the program SigmaStat 3.5 (Systat Software, Inc.) to test whether the abundance of specific mesozooplankton groups changed over time and with increasing CO_2 levels. Effects of time and pCO_2 were considered significant at $p < 0.05$.

3 RESULTS

3.1 Zooplankton abundance and biomass

At the start of the CO_2 perturbation experiment (t_{-1}), total mesozooplankton abundance in the nine mesocosms ranged between 14,726 individuals m^{-3} in M5 (initially 1400 µatm) and 19,645 individuals m^{-3} in M2 (280 µatm) while the biomass varied between 250 mg m^{-3} (M1/840 µatm) and 359 mg m^{-3} (M8/560 µatm). Over the course of the experiment, the zooplankton abundance decreased in all nine mesocosms to between 6,685 (M9/3000 µatm) and 16,588 individuals m^{-3} (M3/390 µatm) (Fig. 1a). In M2/280 µatm, M6/390 µatm, M8/560 µatm, M7/2000 µatm and M9/3000 µatm, the decrease was statistically significant (Spearman rank test (SR), $-0.943 \leq$ correlation coefficient $r \leq -0.886$, $0.017 \leq p \leq 0.033$). The biomass did not change significantly throughout the experiment (SR, $p > 0.05$) (Fig. 1b).

Total zooplankton abundance was negatively correlated with the CO_2 concentration on t_{25} (SR, $r = -0.695$, $p = 0.030$). On all other days, the Spearman rank coefficient was negative, but not significantly. On t_{19} and t_{25}, the biomass in M9 (3000 µatm) was lower than in the other mesocosms (t_{25}: 195 mg m^{-3} as compared to 270 – 420 mg m^{-3}), however, there were no significant correlations between biomass and CO_2 on any of the six sampling days.

The sediment traps in the mesocosms collected between 4,339 (M7/2000 µatm, t_0-t_1) and 62,826 zooplankton organisms 48 h^{-1} (M5/1400 µatm, t_{28}-t_{29}) (Fig. 1c). The total zooplankton export during the entire experiment varied from 287,507 individuals (M4/280 µatm) to 328,613 individuals (M1/840 µatm), and there were no significant correlations between the number of zooplankton organisms collected in the traps and the initial pCO_2 in the mesocosms on any sampling day.

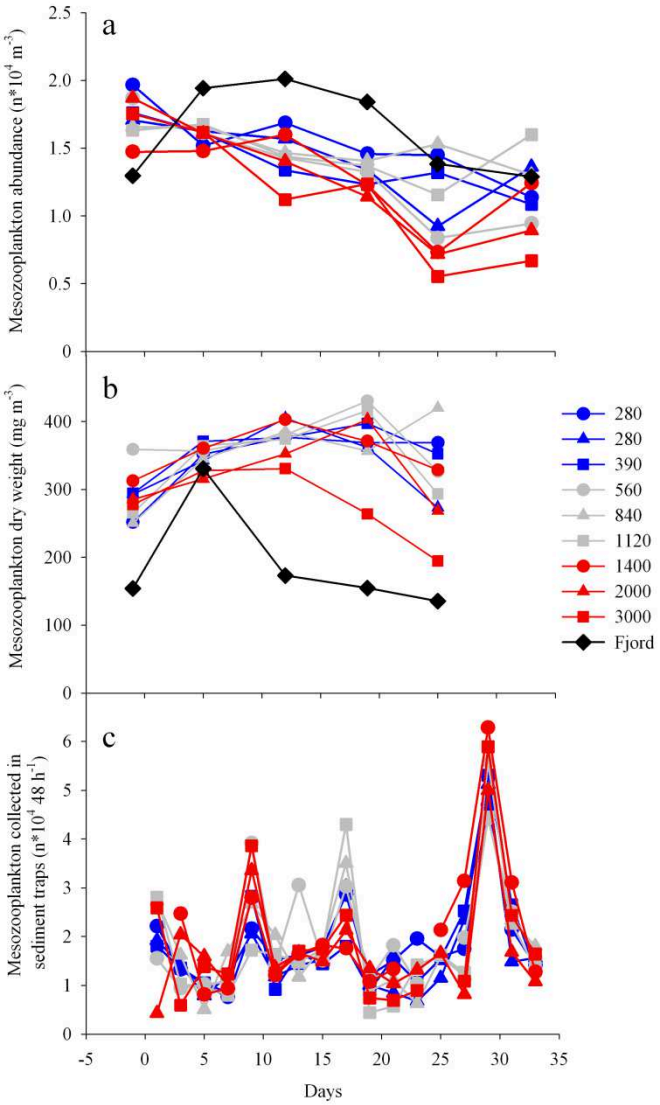

Fig. 1 Mesozooplankton abundance (a) and biomass (b) in the water column of nine mesocosms and in the fjord, and mesozooplankton collected in the sediment traps of the mesocosms (c). Numbers in the legend present initial CO_2 concentrations (µatm) after the CO_2 manipulation was completed.

In the fjord, the abundance ranged between 12,964 and 20,105 individuals m^{-3} throughout our study and was very similar to the initial abundances in the mesocosms (Fig. 1a). The zooplankton biomass, however, was comparably low in the fjord, generally ranging between 135 and 173 mg m^{-3} (Fig. 1b). Only on t_5, the biomass (331 mg m^{-3}) was in the same range as that in the mesocosms.

3.2 Zooplankton community composition

The mesozooplankton community was dominated by copepods, contributing 77 - 93 % of the organisms in the water column (Fig. 2a-c), 25 - 97 % of the organisms collected

in the sediment traps (Fig. 3a, b) and 60 - 80 % of the organisms in the fjord (Fig. 4a-c). Bivalve and gastropod larvae were also found in all water samples, contributing 1 - 36 % and 0.5 - 25 % of the non-copepod community, respectively (Fig. 2d). Cirripeds occurred in all mesocosm water samples but one (Fig. 2d) and also bryozoans contributed a large part (maximum 61 %) of the non-copepod zooplankton community in the water column. Appendicularians were only irregularly found during the first half

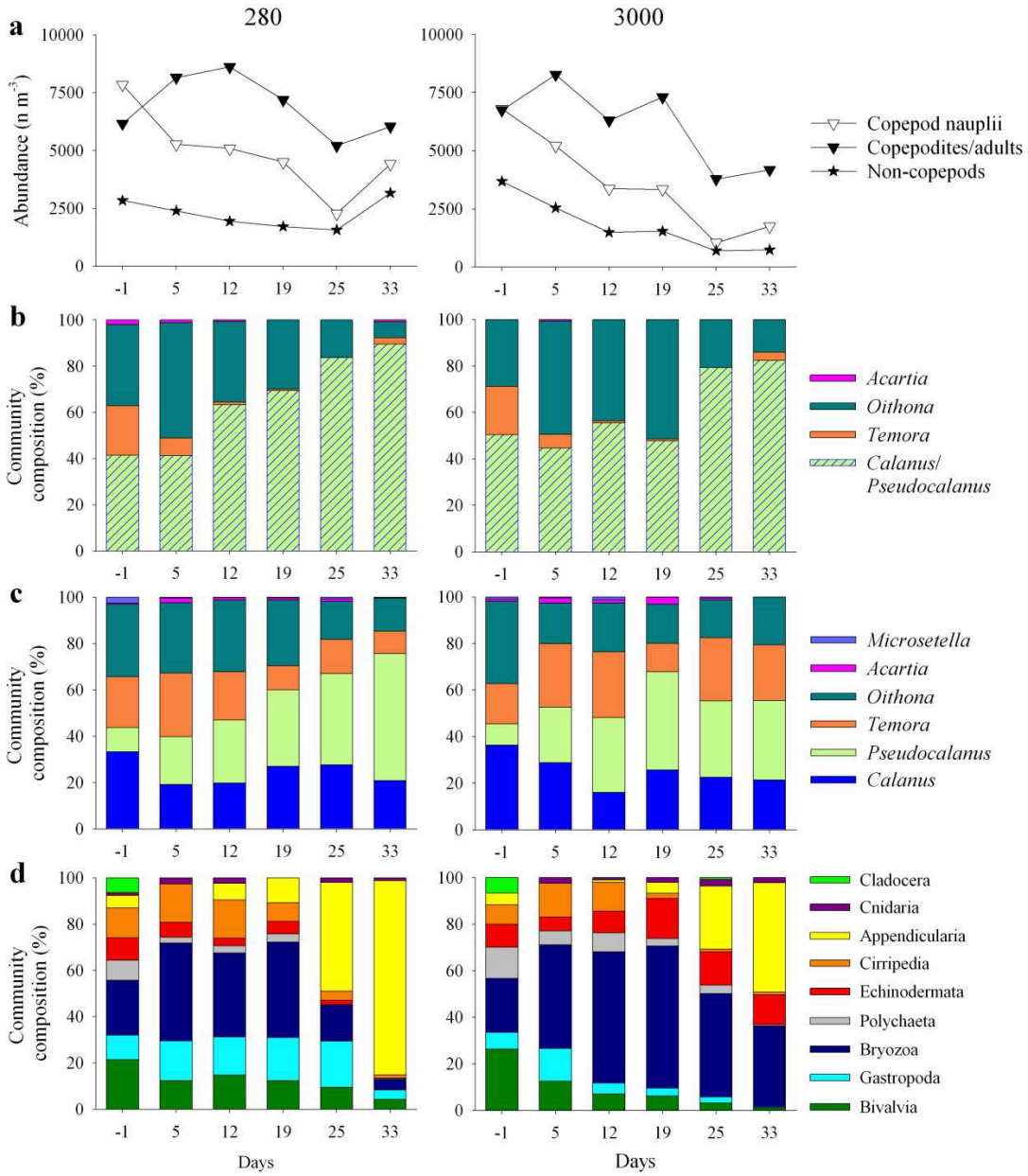

Fig. 2 Mesozooplankton in the water column of the mesocosms: abundance (a) and community composition of copepod nauplii (b), copepodites and adult copepods (c) and non-copepod groups (d) in M4/280 µatm (left side) and M9/3000 µatm (right side).

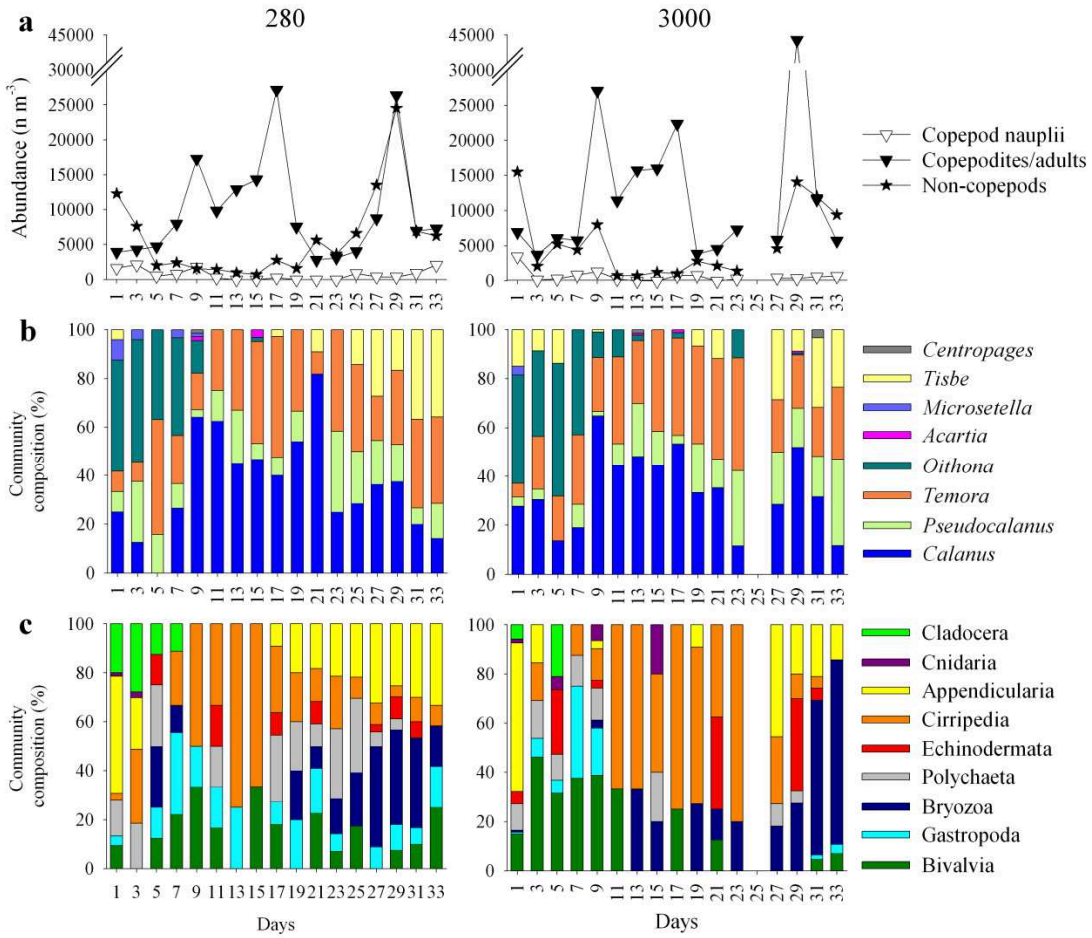

Fig. 3 Mesozooplankton collected in the sediment traps of the mesocosms: abundance (a) and community composition of copepod nauplii (b), copepodites and adult copepods (c) and non-copepod groups (d) in M4/280 µatm (left side) and M9/3000 µatm (right side).

of the study, but increased in abundance thereafter. Polychaete and echinoderm larvae as well as cnidarians were found in most samples, but at relatively low numbers, while cladocerans were mainly found at the start of the study (Fig. 2d). Other mesozooplankton groups (e.g. decapods, chaetognaths and phoronid larvae) were very rare (<1% of the community) and thus excluded from the following analyses. As abundance and community composition generally did not vary much among mesocosms, only the mesocosms with control pCO_2 (M4/280 µatm) and the highest pCO_2 (M9/3000 µatm) are shown in the figures. Results from all nine mesocosms are presented in the supplement.

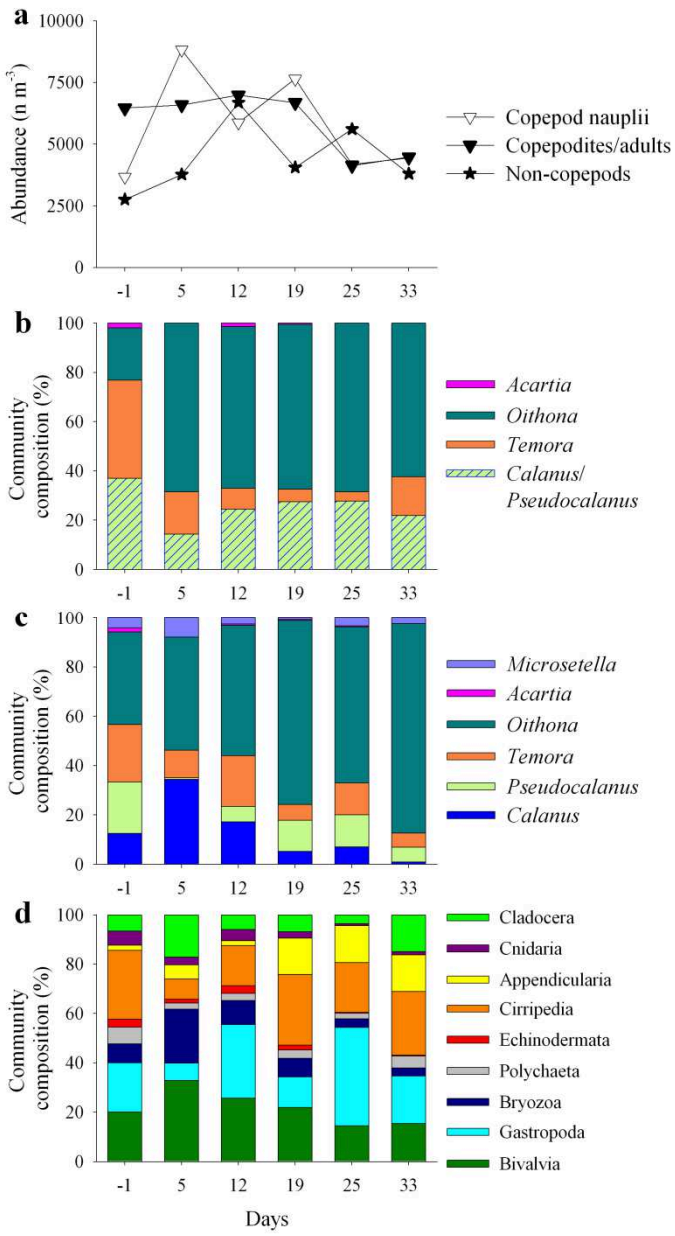

Fig. 4 Mesozooplankton in the fjord: abundance (a) and community composition of copepod nauplii (b), copepodites and adult copepods (c) and non-copepod groups (d).

The fjord community was very similar to those in the mesocosms at the first sampling day. While the community composition in the mesocosms changed of time, that of the fjord remained comparably unchanged (Fig. 4). Here, cirripedia were found throughout the study period and mollusc larvae always contributed >30% to the non-copepod fraction.

The zooplankton diversity in the mesocosms is presented as Shannon index H. An equal occurrence of all ten groups would result in $H = l_n(10)$, i.e. 2.3. Due to the dominance of copepods, however, species diversity was low (< 1) in the mesocosms (Fig. 5a). LME of

the time course of the H index within the water column show that H decreased over time. No significant two- and three-way interactions between Day, Nutr and CO_2 were found, however, to allow for a visually better fit, we kept the effect of Day*Nutr in the depicted model response (colored lines). CO_2 also had no significant effect on the diversity, but was plotted to see the ordering of low, medium and high CO_2 groups.

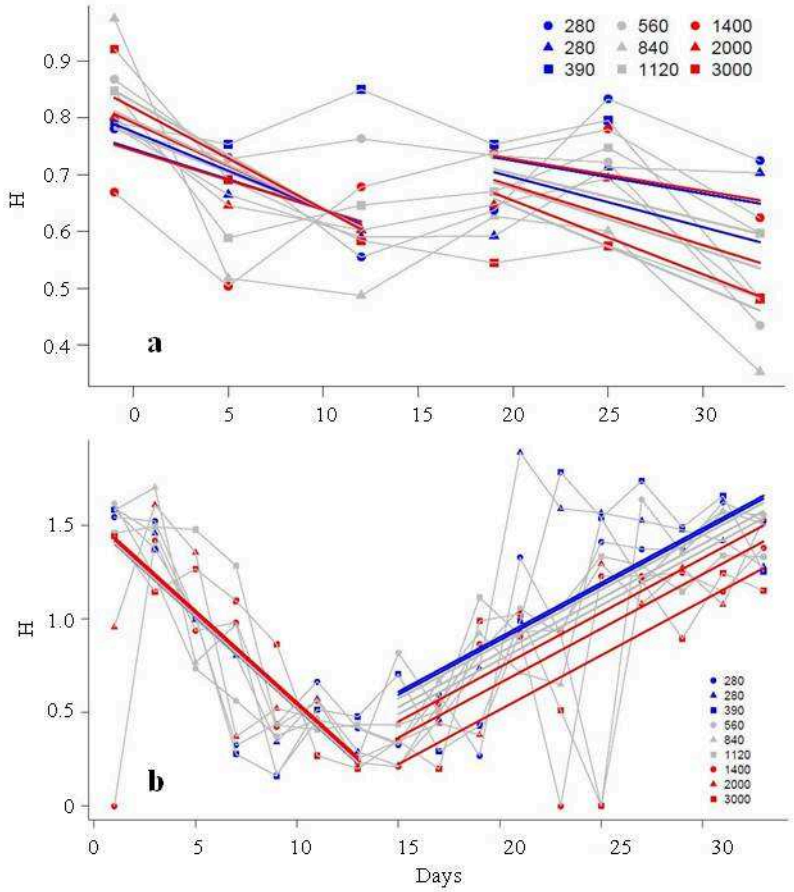

Fig. 5 Mesozooplankton diversity (calculated as Shannon index H) in the water column (a) and the sediment traps (b) of nine mesocosms. Numbers in the legend present initial CO_2 concentrations (µatm) after the CO_2 manipulation was completed. Linear mixed effect models (colored lines) were fitted to determine the dependence of H on time, Nutr and CO_2 (please see text).

In the sediment traps, Nutr significantly affected the zooplankton diversity (Fig. 5b). During the first bloom phase (< day 14), H decreased at all CO_2 concentrations, whereas it increased again after nutrients were added (> day 14). CO_2 itself had significant effects on the diversity. Before nutrient addition, the diversity was slightly higher in high as compared to low CO_2 mesocosms, whereas after nutrient addition, H was considerably lower at high pCO_2.

3.3 Copepods

3.3.1 Abundance

The copepod community in the mesocosms (Fig. 2b, c) and in the sediment traps (Fig. 3b) was dominated by the calanoid species *Calanus finmarchicus*, *Temora longicornis* and *Pseudocalanus elongatus* as well as by *Oithona similis* (Cyclopoida). *Acartia* spp. (Calanoida) and *Microsetella* spp. (Harpacticoida) occurred regularly in the water column of the mesocosms, their abundances, however, were low, and in the sediment traps, they were only found in few samples. *Tisbe* spp. (Harpacticoida), in contrast, were rare in the water column, but occurred in high numbers in the sediment traps. In the fjord, *O. similis* dominated the copepod community throughout the sampling period, whereas *C. finmarchicus*, *T. longicornis* and *P. elongatus* were found less frequently. Other copepod species were rare (Fig. 4b, c).

At the beginning of the experiment, nauplii and copepodites were equally abundant in the mesocosms. With time, the number of nauplii decreased, and copepodite stage I-V and adults (summarized as copepodites) made up the largest part of the copepod community (Fig. 2a). Most nauplii were determined to species level, however, it was not possible to differentiate between the nauplii of *C. finmarchicus* and *P. elongatus*. They were thus treated as one group. *C. finmarchicus*/*P. elongatus* nauplii made up 12 to 38 % of the copepod community in the water column of the mesocosms. Total abundances of these nauplii ranged between 828 and 4,965 ind. m^{-3} and did not change over time, except for M9/3000 µatm, in which nauplii numbers decreased significantly (SR, r = -0.943, p = 0.017). On t_{19} and t_{25}, the abundance of *C. finmarchicus*/*P. elongatus* nauplii was negatively correlated to the CO_2 concentration (SR, t_{19}: r = -0.912, $p < 0.001$; t_{25}: r = -0.728, r = 0.020) (Fig. 6a). The sediment traps collected on average between 394 (M8/560 µatm) and 694 individuals (M1/840 µatm) 48 h^{-1} throughout the experiment. Sampling date and pCO_2 had no effect on the number of nauplii sinking out of the water column, except for the first two sampling days, when more nauplii were found in the sediment traps of the high as compared to the low CO_2 mesocosms (SR, r = 0.731, p = 0.029).

C. finmarchicus copepodite abundances in the mesocosms initially ranged between 1,839 (M1/840 µatm) and 3,065 ind. m^{-3} (M8/560 µatm) and decreased with time to 896

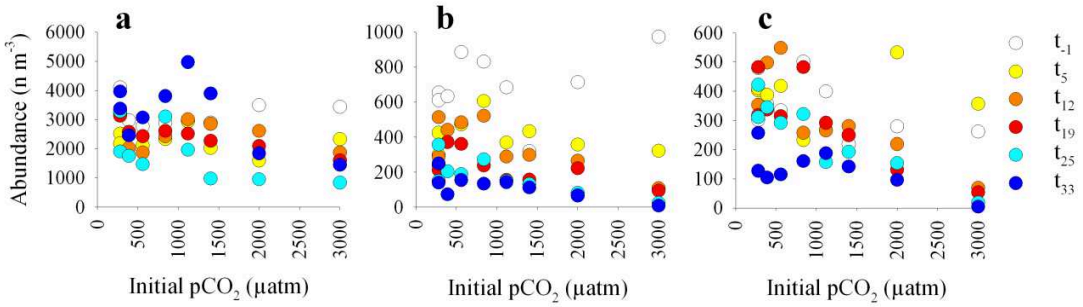

Fig. 6 Abundance of *Calanus finmarchicus*/*Pseudocalanus elongatus* nauplii (a), bivalve larvae (b) and gastropod larvae (c) from nine mesocosms with different seawater pCO_2 on different sampling days (t).

(M9/3000 µatm) - 2,023 ind. m^{-3} (M3/1120 µatm). The decrease was significantly, however, only in M8/560 µtm, M5/1400 µatm, M7/2000 µatm and M9/3000 µatm (SR; $-0.886 \geq r \geq -1.000$; $0.033 \geq p \geq 0.003$). The sediment traps collected on average between 4,011 and 6,245 ind. 48 h^{-1} over the course of the experiment. CO_2 did not affect *C. finmarchicus* abundances, neither in the water column nor in the sediment traps, except for two days (SR, water column: t_{25}, $r = -0.744$, $p = 0.016$; sediment trap: t_{29}, $r = 0.887$, $p < 0.001$). Overall, *C. finmarchicus* copepodites and adults contributed 9 to 22 % to the copepod community in the water column and 0 to 82 % to that in the sediment traps.

P. elongatus copepodites contributed only a small part to the copepod community at the beginning of the experiment (3 to 10 %) with abundances between 398 (M1/840 µatm) and 1471 ind. m^{-3} (M8/560 µatm). Their numbers, however, increased to 1,425 (M9/3000 µatm) and 4,107 ind. m^{-3} (M1/840 µatm), thus contributing 24 to 34 % of all copepods at t_{33}. In four mesocosms, the abundance was significantly related to sampling day (SR, M2/280 µatm, M6/390 µatm, M1/840 µatm and M3/1120 µatm; $0.886 \leq r \leq 1.000$, $0.033 \geq p \geq 0.003$). Also in the sediment traps, the number of *P. elongatus* copepodites increased with time (SR, significant in M6/390 µatm, M3/1120 µatm, M5/1400 µatm and M7/2000 µatm, $0.513 \leq r \leq 0.694$, $0.048 \geq p \geq 0.002$). Seawater pCO_2 did usually not affect the abundance of *P. elongatus*. Only on t_{25}, copepods numbers were significantly lower at elevated pCO_2 (SR, $r = -0.762$, $p = 0.012$).

T. longicornis contributed 5 to 24 % of the copepod community in water columns of the mesocosms. Total abundances on t_{-1} ranged from 1165 (M8/560 µatm) to 1839 nauplii m^{-3} (M7/2000 µatm) and from 674 (M2/280 µatm) to 1839 copepodites m^{-3} (M6/390

µatm). With time, nauplii abundances decreased to less than 150 ind. m^{-3}. The abundance of copepodites did not change significantly. In the sediment traps, copepodites of *T. longicornis* were present in high numbers (2,783 (M3/1120 µatm) - 3,870 ind. 48 h^{-1} (M8/560 µatm)) while nauplii were only found in 28 of 153 samples. CO_2 had no influence on the abundance of *T. longicornis*, neither in the water column nor in the sediment traps (SR, $p > 0.05$).

O. similis was found in high numbers, ranging from 3,249 (M5/1400 µatm) to 5,884 ind. m^{-3} (M2/280 µatm), in the mesocosms at the beginning of the experiment. With time, abundances decreased to 858 (M8/560 µatm) - 2,237 ind. m^{-3} (M3/1120 µatm) at t_{33}. In all mesocosms except for M1/840 µatm and M5/1400 µatm, the decrease was significant (SR; $-0.886 \geq r \geq -1.000$, $0.033 \geq p \geq 0.003$). The sediment traps collected between 2,009 (M3/1120 µatm) and 2,781 ind. 48 h^{-1} (M9/3000 µatm) during the first 10 days, while *O. similis* was rarely found during the last three weeks of the experiment. The seawater pCO_2 did not affect *O. similis* abundances in the water column and the sediment traps, except for t_{19}, when copepod numbers decreased with increasing pCO_2 (SR, $r = -0.717$, $p = 0.025$).

Acartia spp. occurred in most samples from the mesocosm water column, but its abundance did not exceed 582 ind. m^{-3} and, thus, it never contributed more than 4 % of the copepod community. At the first sampling day, *Acartia* nauplii outnumbered the copepodites. With time their abundance decreased (significantly in M2/280 µatm, M1/840 µatm, M5/1400 µatm and M7/2000 µatm; SR, $-0.845 \geq r \geq -0.971$, $0.033 \geq p \geq 0.003$). The copepodite abundance did not change. In the sediment traps, *Acartia* spp. were only found in 16 of 153 samples, with a maximum of 462 individuals 48 h^{-1}. CO_2 did not significantly affect the abundance of *Acartia* spp.

Similar to *Acartia* spp., copepodites of *Microsetella* spp. were also found regularly in the mesocosms, but in low numbers ($\leq$ 368 ind. m^{-3}). Nauplii were rare. In the sediment traps, *Microsetella* spp. were only found in few samples ($\leq$ 540 ind. 48 h^{-1}) and mainly during the first half of the experiment. The seawater pCO_2 had no influence on *Microsetella* spp. abundances.

Tisbe spp. were rare in the mesocosms, however, frequent in the sediment traps (≤ 7442 ind. 48 h^{-1}), especially during the second half of the experiment. CO_2 did generally not affect the abundance of *Tisbe* spp., except for t_{19}, when copepod numbers in the sediment traps increased with increasing pCO_2 (SR, r = 0.766, p = 0.012).

3.3.2 Stage development

Nauplii and copepodite developmental stages were determined for the most abundant copepod species in the mesocosms, i.e. *C. finmarchicus/P. elongatus* (not distinguished), *T. longicornis*, and *O. similis*, to detect possible effects of elevated seawater pCO_2 on the copepod development. In *Calanus/Pseudocalanus* nauplii (Fig. 7a), all developmental stages were present throughout the experiment in almost equal shares indicating continuous production of young nauplii, and thus egg production. A similar pattern was found in *P. elongatus* copepodites (Fig. 7c). In *C. finmarchicus*, in contrast, early copepodite stages disappeared during the experiment, and most copepods had developed to copepodite stage V at the last sampling day (Fig. 7b). In *O. similis* and *T. longicornis*, there was also no marked developmental succession visible, and nauplii and copepodite stages as well as adults were present throughout the experiment (Fig. 7e-g). In the fjord, the developmental patterns were the same as in the mesocosms. CO_2 did not have an effect on development in any of the species.

3.4 Bivalves

Initial bivalve abundances ranged between 318 ind. m^{-3} (M3/1120 µatm) and 973 ind. m^{-3} (M9/3000 µatm). Over the course of the experiment, their abundances decreased significantly in all mesocosms (SR, $-0.943 \geq r \geq -1.00$, $0.017 \geq p \geq 0.003$), except for M2/280 µatm (SR, r = -0.771, p = 0.103), and on t_{33}, 8 (M9/3000 µatm) - 249 ind. m^{-3} (M2/280 µatm) were found. CO_2 significantly affected bivalve larvae abundances (Fig. 6b). Until t_5, the day after which the CO_2 manipulation was completed, no correlation between bivalve abundance and the seawater pCO_2 was found. On t_{12} and t_{19}, bivalve numbers decreased, however not statistically significant, with increasing CO_2 concentrations (SR, r = -0.601, p = 0.077). From t_{25} on, the bivalve abundance decreased significantly with increasing pCO_2 (SR, t_{25}: r = -0.786, p = 0.009; t_{33}: r = -0.695, p = 0.030). In the sediment traps, on average 295 (M7/2000 µatm) to 805 bivalve larvae 48 h^{-1} (M8/560 µatm) were collected, with no apparent trend over time. Only in

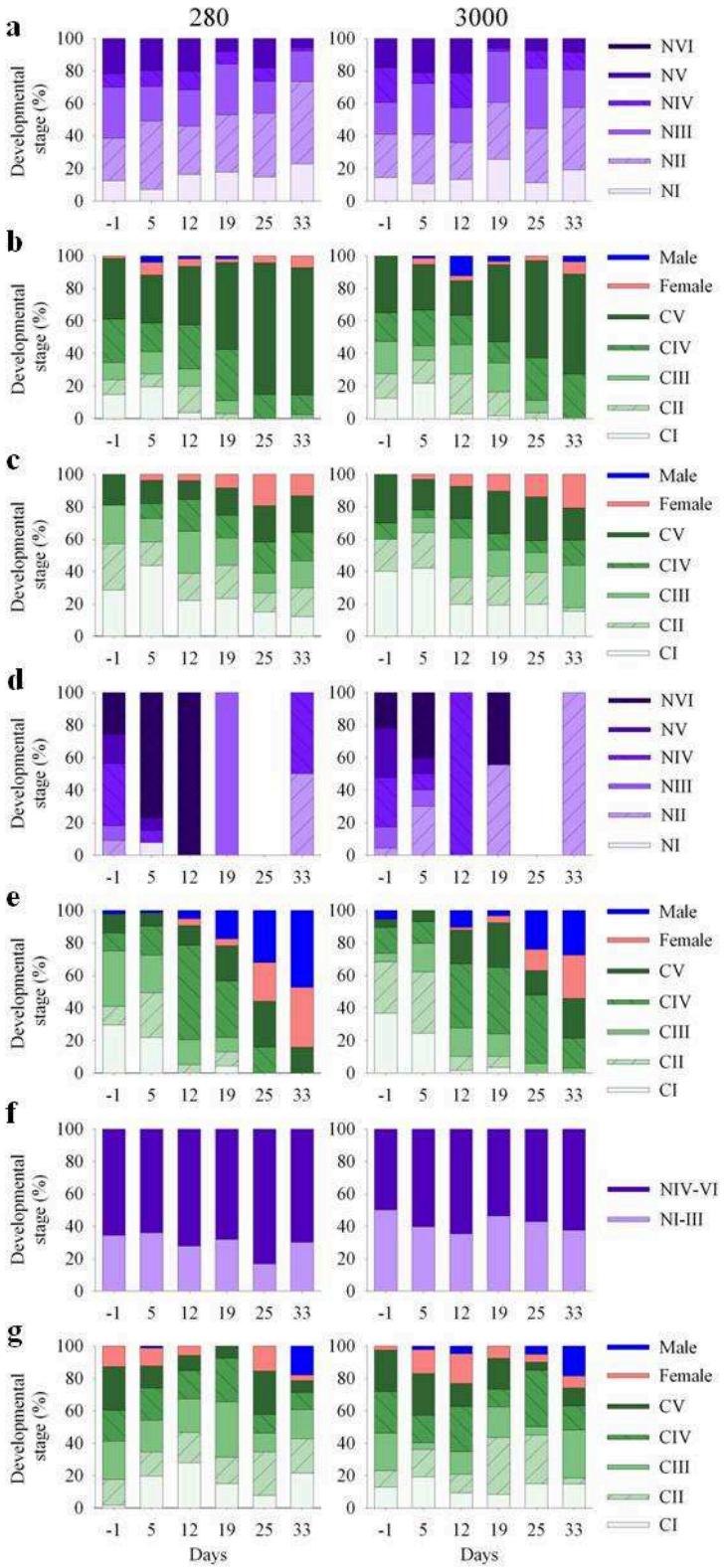

Fig. 7 Copepod stage development in the water column of the mesocosms: *Calanus finmarchicus*/*Pseudocalanus elongatus* nauplii (a), *C. finmarchicus* copepodites/ adults (b), *P. elongatus* copepodites/adults (c), *Temora longicornis* nauplii (d), *T. longicornis* copepodites/ adults (e), *Oithona similis* nauplii (f) and *O. similis* copepodites/adults (g) from M4/280 µatm (left side) and M9/3000 µatm (right side). C = copepodite stage; N = nauplius stage. On day 25, no *T. longicornis* nauplii were found.

M2/280 µatm, abundance increased significantly (SR, r = 0.693, p = 0.002), whereas it decreased in M9/3000 µatm (SR, r = -0.553, p = 0.026). Until t_{11}, the number of bivalves found in the sediment traps increased with increasing pCO_2 levels, however, significantly only on t_9 (SR, r = 0.870, p < 0.001). From t_{17} on, abundances in the sediment traps decreased with increasing CO_2, and significant negative correlations were evident on t_{23} (SR, r = -0.791, p = 0.015) and on t_{29} (r = -0.924, p < 0.001).

In the fjord, bivalve abundances ranged between 552 and 1,716 ind. m^{-3} and were generally higher as compared to the mesocosms. They made up between 14 and 33 % of the non-copepod fjord community (Fig. 4d).

3.5 Gastropods

At the start of the experiment, gastropod abundances varied between 218 ind. m^{-3} (M5/1400 µatm) and 502 ind. m^{-3} (M1/840 µatm). Over time, their abundances decreased significantly in the two mesocosms with the highest pCO_2 (SR, M7/2000 µatm: r = -0.886, p = 0.033; M9/3000 µatm: r = -0.943, p = 0.017). At t_{12}, t_{19} and t_{25}, CO_2 significantly affected gastropod abundance (SR, $-0.879 \leq r \leq -0.778$, $0.001 \leq p \leq 0.009$) (Fig. 6c). On t_{33}, it did not (SR, r = -0.485, p = 0.169). The sediment traps collected on average between 268 ind. (M9/3000 µatm) and 750 ind. 48 h^{-1} (M2/280 µatm). In M6 (390 µatm) and M8 (560 µatm), abundances increased significantly over time (SR, $0.547 \leq r \leq 0.779$, $0.001 \leq p \leq 0.028$). During the first two weeks, CO_2 did not affect gastropod abundances, except for t_9, when abundances increased significantly with pCO_2 (SR, r = 0.727, p = 0.020). From t_{17} on, gastropod abundances decreased with increasing pCO_2, with significant negative correlations on t_{21} (SR, r = -0.723, p = 0.025) and on t_{29} (SR, r = -0.770, p = 0.012). In the fjord, the abundance of gastropods varied strongly among different sampling days, ranging from 261 to 2,237 ind. m^{-3}.

3.6 Cirripedia

At t_{-1}, between 306 (M9/3000 µatm) and 797 ind. m^{-3} (M7/2000 µatm) were found in the mesocosms. More than 80 % of these cirripedia were in the nauplius stage. Over time, their abundance decreased significantly (SR, $-1.000 \leq r \leq -0.886$, $0.003 \leq p \leq 0.033$), except for M1/840 µatm (SR, r = -0.771, p = 0.103), and on the last sampling day, only 8 to 161 nauplii m^{-3} were found. Cypris larvae, the cirriped developmental

stage which finally settles on hard substrate, were rare in the water column throughout the experiment, with abundances ranging between 0 and 153 ind. m^{-3}. On t_{33}, cypris larvae made up 40 to 100 % of all cirripeds.

The sediment traps collected 0 to 2,633 cirripedia nauplii 48 h^{-1} (average: 142 (M3/1120 µatm) - 489 ind. 48 h^{-1} (M7/2000 µatm)). Abundances did not change significantly over time, except for M4 (280 µatm) (SR, $r = -0.582$, $p = 0.014$). Cypris larvae occurred in relatively low numbers in the sediment traps during the first week (143 ± 293 ind. 48 h^{-1}). Throughout the experiment, however, abundances increased, and significant positive correlations between cypris larvae abundance and sampling day were found in M2/280 µatm (SR, $r = 0.757$, $p < 0.001$), M4/280 µatm ($r = 0.489$, $p = 0.046$), M1/840 µatm ($r = 0.703$, $p = 0.001$) and M7/2000 µatm ($r = 0.851$, $p < 0.001$). At maximum, 2,564 larvae 48 h^{-1} were collected.

Nauplii abundance was not significantly affected by the seawater pCO_2. In cypris larvae, there were significant relations between abundance and pCO_2 on a few days, however, with no clear trend (SR, t_{13} (sediment trap): $r = -0.703$, $p = 0.030$; t_{19} (sediment trap), $r = 0.929$, $p < 0.001$; t_{33} (water column), $r = -0.877$, $p < 0.001$).

In the fjord, 306 - 1,165 cirripedia m^{-3} were found during our study. More than 85 % of the cirripeds were in the nauplius stage. From t_{10} on, nauplii abundance was considerably higher than in the mesocosms, reaching a maximum of 1,149 ind. m^{-3}, whereas the abundance of the cypris larvae was as low as in the mesocosms.

3.7 Bryozoa

Bryozoan abundances ranged from 398 (M8/560 µatm) to 1,165 ind. m^{-3} (M5/1400 µatm) at t_{-1} and did not change significantly over time. In the sediment traps, bryozoa were encountered only irregularly throughout the first 23 days of the experiment, and numbers did not exceed 1,583 ind. 48 h^{-1}. During the last ten days, bryozoan numbers increased significantly in all mesocosms (SR, $0.593 \leq r \leq 0.905$, $0.001 \leq p \leq 0.012$). At maximum, 11,799 individuals 48 h^{-1} were collected (t_{29}, M6/390 µatm). CO_2 did generally not affect the abundance of bryozoans in the water column and in the sediment traps, except for t_{27}, when numbers decreased with increasing pCO_2 (SR, r =

-0.854, $p < 0.001$). In the fjord, bryozoan abundances were comparable to those in the mesocosms, ranging between 123 und 828 ind. m^{-3}.

3.8 Appendicularia

During the first three weeks of the experiment, appendicularian abundances in the mesocosms were low with 0 to 184 ind. m^{-3}. Thereafter, numbers increased, although significantly only in M6/390 µatm (SR, $r = 0.899$, $p = 0.017$) and M7/2000 µatm (SR, $r = 0.986$, $p = 0.003$). On t_{33}, between 199 and 2,651 ind. m^{-3} were found in the water column. In the sediment traps, between 372 and 11,532 ind. were collected between t_0 and t_1. Until t_7, abundances decreased considerably, and between t_7 and t_{15}, no appendicularians were found in most of the traps. Maximum abundances were 628 ind. 48 h^{-1} during this time. Thereafter, in accordance to the development in the water column, appendicularian numbers started to increase again and peaked on t_{29} in almost all mesocosms, ranging between 2,821 (M9/3000 µatm) and 14,104 ind. 48 h^{-1} (M5, 1400 µatm). Neither in the water column nor in the sediment traps, appendicularian abundances were significantly affected by the seawater pCO_2. In the fjord, appendicularian numbers ranged between 61 and 843 ind. m^{-3}. Similar to those in the mesocosms, they increased slightly, but non-significantly, in abundance over time.

3.9 Polychaetes

Polychaete larvae were rare in the water column of the mesocosms, generally not exceeding 200 ind. m^{-3}. Highest abundances were found at the start of the experiment, with 92 (M3/1220 µatm) to 490 ind. m^{-3} (M7/2000 µatm). Over time, polychaete larvae decreased in abundance, however, significantly only in four of the nine mesocosms (SR, M4/280 µatm: $r = -0.880$, $p = 0.033$; M1/840 µatm: $r = -1.000$, $p = 0.003$; M7/2000 µatm: $r = -0.943$, $p = 0.017$; M9/3000 µatm: $r = -1.000$, $p = 0.003$). On the last sampling day, 0 to 15 ind. m^{-3} were found. The sediment traps collected between 0 and 2,956 ind. 48 h^{-1}, with an average of 343 (M9/3000 µatm) to 728 individuals 48 h^{-1} (M1/840 µatm). In M9/3000 µatm, polychaetes increased in abundance over time (SR, $r = 0.503$, $p = 0.046$). In the other mesocosms, the sampling day did not affect abundances. Elevated seawater pCO_2 did not influence polychaete abundances, neither in the water column nor in the sediment traps. In the fjord, polychaete larvae were also found only in low numbers, ranging between 92 and 184 ind. m^{-3} throughout the sampling period.

3.10 Echinodermata

Echinoderm larvae were regularly found in the mesocosms, although total abundances were low and did not exceed 400 ind. m^{-3}, Over time, abundances decreased, significantly however only in the two control mesocosms (SR, M2: r = -0.886, p = 0.033; M4: r = -0.943, p = 0.017). At the last day, 8 (M8/560 µatm) to 96 ind. m^{-3} (M9/3000 µatm) were found. In the sediment traps, echinoderms occurred only irregularly, and abundances rarely exceeded 1,000 ind. 48 h^{-1}. Higher numbers of larvae were, however, found on t_{29}, ranging between 1443 (M2/280 µatm) and 5290 ind. 48 h^{-1} (M9/3000 µatm). On average, 254 (M4/280 µatm) to 569 ind. 48 h^{-1} (M5, 1400 µatm) were collected. CO_2 did generally not affect the abundance of echinoderms. Only on t_{19}, abundance in the water column increased with increasing pCO_2 (SR, r = 0.735, p = 0.020). In the fjord, echinoderms also made up only a very small percentage of the community (Fig. 4d). Abundances varied between 15 and 215 ind. m^{-3} and were therefore similar to those in the mesocosms.

3.11 Cnidaria

Cnidarian medusae were rare in all mesocosms (Fig. 2d, 3c). In the water column, their abundances did not exceed 153 ind. m^{-3} In the sediment traps, cnidaria were seldom found, and at maximum 515 individuals sank down within 48 h. On t_5, abundances increased with increasing pCO_2 (SR, r = 0.733, p = 0.020), however, on none of the other sampling days, a correlation between abundance and CO_2 was evident. In the fjord, cnidarian abundance was generally similar to that in the mesocosms (46 to 153 ind. m^{-3}). Only on t_{12}, a higher abundance (306 ind. m^{-3}) was found.

3.12 Cladocera

Cladocerans (mainly *Evadne* spp. and *Podon* spp.) were found mainly during the first week of the experiment (Fig. 2d, 3c). In the water column, initial cladoceran abundances ranged between 184 (M4/280 µatm) and 368 ind. m^{-3} (M3/1120 µatm). Thereafter, they were only found rarely, with maximum numbers of 61 ind. m^{-3}. In the sediment traps, on average 495 (M7/2000 µatm) to 1,588 individuals 48 h^{-1} (M2/280) were collected throughout the first week of the experiment. Thereafter, no cladocerans were found anymore. CO_2 did not affect abundance, except for one day (sediment trap,

t_1: SR, r = -0.898, $p < 0.001$). In the fjord, cladocerans were found constantly throughout the sampling period (Fig. 4d), and abundances ranged between 184 and 644 ind. m^{-3}.

4 DISCUSSION

Large-scale mesocosms are valuable tools to study the effects of OA on zooplankton community dynamics (Riebesell et al. 2010). In such enclosures, organisms of at least three trophic levels in naturally occurring proportions can be kept at close to natural, self-sustaining environmental conditions, providing the opportunity to sample the same population repeatedly over an extended period of time (Riebesell et al. 2010). However, sampling the mesocosms with plankton nets, as in our study, alters the zooplankton abundance in the enclosures and therefore their impact on other planktonic organisms. Similar to the study at Svalbard (Niehoff et al. 2013, Riebesell et al. 2013), the total number of all net hauls was thus restricted to approx. one-sixth of the total cross-sectional area of the mesocosms, and as zooplankton was also sampled for other studies (Hildebrandt et al. in press, Hildebrandt and Niehoff, unpubl. data, Büdenbender et al. unpubl. data), it was not possible to take replicate samples for analyzing the community development. Rare zooplankton groups such as decapods, chaetognaths and phoronid larvae were thus likely not sampled quantitatively and therefore excluded from the analyses.

A prerequisite for mesocosm studies is that the initial conditions are identical in all enclosure bags. In our study, parameters such as nutrient and chlorophyll *a* concentrations and the phytoplankton community composition were similar among mesocosms at the start of the experiment (K. G. Schulz, unpubl. data). Also the mesozooplankton biomass, abundance and community composition did not vary much among the nine bags at the time of closure, prior to the CO_2 manipulation, and no significant correlations between the abundance of zooplankton groups and the target pCO_2 levels of the mesocosms at t_{-1} were found, which would have biased the community response to OA throughout the study.

In the fjord, the zooplankton community composition at the start of our study was similar to that in the mesocosms. Over the time of the experiment, however, the communities in the fjord and in the control mesocosms developed differently, likely

owing to the nutrient addition in the mesocosms as well as lacking advection and interactions with top-predators and the benthos. The community development in the mesocosms does thus not completely mirror natural conditions, but due to the identical starting conditions, the mesocosm communities can well be compared among each other.

The CO_2 manipulation resulted in initial pCO_2 values of 280 to 3000 µatm and corresponding pH values of 8.2 to 7.2. Over time, however, the pCO_2 decreased due to outgassing and the uptake of CO_2 by phytoplankton organisms, especially in the surface layers (K. G. Schulz, unpubl. data). It has thus to be noticed that the CO_2 response of organisms in a distinct mesocosm does not represent the response to a particular pCO_2 level, but to a continuously changing pCO_2. However, there was still a gradient in pCO_2 and pH among the mesocosms at the end of the experiment, allowing to study the effects of elevated pCO_2 on a natural mesozooplankton community.

Ten zooplankton groups, i.e. copepods, bivalves, gastropods, cirripeds, echinoderms, polychaetes, bryozoans, appendicularians, cladocerans and cnidarians, contributed regularly to the community in the mesocosms, i.e. they were found in ≥ 35 % of the samples. However, due to the dominance of copepods, which made up 80 to 90 % of all zooplankton organisms, the diversity index H was low. During the experiment it decreased, which likely was caused by the development of the non-copepod zooplankton, as the appendicularian abundance increased, while the abundance of all other groups decreased and they thus contributed less to the community. In most taxonomic groups (classes, genera, species and developmental stage, depending on the taxonomic level determined), abundance in the water column did not relate significantly to the pCO_2. Only in bivalve and gastropod larvae and in nauplii of the copepods *C. finmarchicus* and *P. elongatus* (not distinguished), pCO_2 was negatively related to abundance at the end of the experiment. These effects did not, however, lead to significant differences in the diversity, calculated as Shannon Index H, among the communities, which developed at different pCO_2 in the nine mesocosms. In the sediment traps, a significant positive correlation between pCO_2 and the Shannon index H was found during the first phytoplankton bloom phase, while during the second bloom event a negative correlation was evident. The sediment traps collect all animals which either migrate (active swimmers) or which die and sink towards the bottom. In

the high pCO_2 mesocosms, high numbers of molluscs were collected in the sediment traps during the first half of the experiment, indicating that CO_2 negatively affected the survival of these organisms. Accordingly, their abundance in the water column decreased stronger, and total bivalve and gastropod numbers during the second half were lower in the high pCO_2 mesocosms as compared to the low pCO_2 mesocosms. Therefore, during the second half relatively few individuals sank into the sediment traps of the high pCO_2 mesocosms, simply owing to the low numbers of mollusc larvae in the water column. As no CO_2 effects were found for the other zooplankton groups in the sediment traps, bivalve and gastropod larvae have likely caused the altered diversity index in relation to CO_2.

The gastropods found in the mesocosms in the Raunefjord were presumably a mixture of pteropods (*Limacina* spp.) and benthic gastropod larvae such as *Lunatia intermedia*, *Littorina littorea* and *Gibbula cineraria*, which are common in the Raunefjord (Tunberg 1982). At least 32 bivalve species are described from this area, with *Lucinoma borealis*, *Dosinia exoleta* and *Astarte montagui* being the most abundant ones (Tunberg 1981, 1982), and all of these species might have contributed to the bivalve larvae sampled in the mesocosm. Bivalve larvae are, however, extremely difficult to distinguish and a complete taxonomic analysis was beyond our capacities. Molluscs are, among other calcifying animals, believed to be the most threatened marine organisms in the face of OA (reviews by Kroeker et al. 2013, Wittmann and Pörtner 2013). The decreasing calcium carbonate saturation in the water hampers the shell formation and elicits shell dissolution in most species studied to date, which subsequently can affect survival (e.g. Ries et al. 2009, Watson et al 2009, Comeau et al. 2010, Lischka et al. 2010, Talmage and Gobler 2010, Melatunan et al. 2013). However, OA can also affect other processes in molluscs. The feeding rates of the gastropod *Concholepas concholepas*, for example, were significantly reduced at $pCO_2 \geq 700$ µatm (Vargas et al. 2013). In the intertidal gastropod *Littorina obtusata*, the viability of eggs was reduced, the egg development time increased and the activity of the larvae was significantly lower at 1100 µatm CO_2 as compared to control conditions (Ellis et al. 2009). Thus, the fitness of the species decreases under such conditions, and this contributes to a decline of marine calcifiers (species and individuals) at increasing pCO_2, as reported from natural CO_2 vents and benthic mesocosm studies (e.g. Hall-Spencer et al. 2008, Cigliano et al. 2010, Christen et al. 2013).

Another group of calcifiers present in the mesocosms were echinoderm larvae. Tunberg (1982) found eight species of echinoderms in the Raunefjord, including e.g. the brittle stars *Ophiura albida* and *Ophiocomina nigra*, the seastar *Astropecten irregularis* and the sea urchin *Echinocardium flavescens*. During our experiment, the abundance of echinoderm larvae was not affected by high pCO_2. Recent studies have shown that the survival rates of echinoderm larvae under near-future OA scenarios differ among species. In larvae of the brittle star *Ophiothrix fragilis*, a decrease in pH of 0.2 to 0.4 units significantly affected the survival (Dupont et al. 2008). In contrast, in four species from tropical (*Tripneustes gratilla*), temperate (*Pseudechinus huttoni*, *Evechinus chloroticus*) and polar regions (*Sterechinus neumayeri*), mortality was not affected at pH levels > 7.0 (Clark et al. 2009). However, elevated pCO_2 levels can induce changes in the scope for growth (Stumpp et al. 2011) and lead to sublethal effects including reduced body size and calcification (e.g. Clark et al. 2009, Sheppard Brennand et al. 2010, Suwa et al. 2013), malformations (Dupont et al. 2008) and decreased digestive efficiency (Stumpp et al. 2013). As an altered scope for growth might especially impact animals when food is limited, it is possible that food for the larvae in the mesocosms was sufficient to cope with OA. Sublethal effects could not be addressed in our study, and they might still affect the echinoderm community in the longer term, both in the pelagic zone (larvae) as well as in the benthos (juveniles and adults).

Copepods dominated the mesozooplankton community in the mesocosms, a prominent feature of especially polar waters and mid-latitude continental shelves (Longhurst 1985). The copepod species, mainly calanoids (*C. finmarchicus*, *T. longicornis*, *P. elongatus*) and cyclopoids (*O. similis*), which we found, are common in the surface waters of the Raunefjord (Matthews 1967, Vestheim et al. 2012). We were not able to differentiate between *Calanus* and *Pseudocalanus* nauplii, however, life cycle strategies and stage development patterns suggest that most nauplii belonged to *P. elongatus*. *P. elongatus* increased in abundance throughout the experiment, and there was no pronounced succession in developmental stages, indicating that the copepods reproduced continuously in the mesocosms. This is supported by the occurrence of females carrying eggs (Hildebrandt and Niehoff, unpubl. data), and it also matches the pattern of the naupliar development stages, i.e. the continuously high proportion of young nauplius stages NI-NIII. *C. finmarchicus* copepodites, in contrast, developed to

late copepodite stages over time, while young copepodite stages disappeared. Also, the number of spawning *C. finmarchicus* females was very low in all mesocosms throughout the study (Hildebrandt and Niehoff, unpubl. data). This finding is not surprising, as reproduction in this species is correlated to the spring phytoplankton bloom (e.g. Niehoff et al. 1999, Niehoff and Hirche 2000), and our experiment started later in the season, during the post-bloom period. *P. elongatus,* on the other hand, can also produce eggs at high rates when chlorophyll *a* content is low, probably by feeding on microzooplankton and detritus (Renz et al. 2008).

Abundance of copepodites and adult copepods and stage composition were generally not affected by elevated CO_2 concentrations. Our mesocosm study thus contributes to the picture that copepods are relatively robust to near-future OA (e.g. Kurihara et al. 2004, Mayor et al. 2012, Troedsson et al. 2012, Weydmann et al. 2012, Niehoff et al. 2013, Hildebrandt et al. in press). However, the abundance of *Calanus*/*Pseudocalanus* nauplii decreased significantly at increasing pCO_2. If we assume that the nauplii belong mostly to *P. elongatus,* this suggests that hatching and nauplii development of this species were impaired by elevated pCO_2. Studies on the response of *Pseudocalanus* spp. to OA are, however, to our knowledge not yet available while *C. finmarchicus* has been studied in greater detail. The egg production rates of this species were not affected by a pCO_2 of 8000 µatm, whereas the hatching success decreased at such high CO_2 concentrations (Mayor et al. 2007). At 3300 µatm, however, survival rates of copepods raised from eggs to adults did not differ from those of copepods kept at control conditions (Pedersen et al. 2013), indicating that pCO_2 levels predicted to occur in the next centuries do not affect the reproduction of *C. finmarchicus*. These results support our hypothesis that the effects of CO_2 on the *Calanus/Pseudocalanus* nauplii group were mainly caused by *P. elongatus*.

Similar to previous studies on *Acartia pacifica*, *A. tsuensis, A. clausi* and *Centropages typicus* (Kurihara and Ishimatsu 2008, Zhang et al. 2011, McConville et al. 2013, Zervoudaki et al. 2014), *Acartia* and *Centropages* spp. in our study were not affected. In contrast, the reproduction of *A. spinicauda* and *C. tenuiremis* is hampered at a $pCO_2 \geq$ 2000 µatm (Zhang et al. 2011). *Tisbe* spp. were insensitive to OA in the mesocosms, whereas nauplii production in the benthic species *Tisbe battagliai* decreases already at a moderate pH of 7.8 (Fitzer et al. 2012).

Another dominant group in the mesocosms were the larvae of cirripeds, such as *Semibalanus balanoides* (*Balanus balanoides* s.l.), which commonly inhabit rocky bottoms in the surrounding of Espegrend (Nelson, 1982). Cirripedia nauplii and cypris larvae can be abundant in the water column in spring (Nejstgaard et al., 2006), and at the beginning of our mesocosm study, nauplii dominated the cirriped developmental stages in the water column. Over time, these nauplii developed to cypris larvae, which settle on hard substrate. Thus, they sank out of the water column and were mainly found in the sediment traps, a pattern that was also reported from the Arctic mesocosm study by Niehoff et al. (2013). Neither cirriped nauplii nor cypris larvae were affected by elevated CO_2 concentrations during the 30 day experiment. This matches results on cirriped survival from the Arctic mesocosm study (Niehoff et al. 2013) as well as laboratory studies at low seawater temperatures (Findlay et al. 2008, 2010, Pansch et al. 2012), however, OA effects appear when temperatures increase (Findlay et al. 2010, Pansch et al. 2012) and thus might alter the community structure in future decades.

Bryozoan larvae contributed a large part (up to 61 %) to the non-copepod zooplankton community in the mesocosms. CO_2 effects on their abundance were not found throughout the study period. To our knowledge, there have been no other studies investigating the response of these cyphonautes larvae to OA, however, bryozoan colonies, similar to other calcifying marine organisms, can be affected. In colonies of *Myriapora truncata* and *Schizoporella errata* which were transplanted to high CO_2 water at a natural vent site, calcification was significantly reduced as compared to control conditions, and skeletal dissolution increased during a long-term exposure (Rodolfo-Metalpa et al. 2010, Lombardi et al. 2011a,b). Also the chemical composition of the skeleton can be altered (Lombardi et al. 2011a), and in *S. errata*, the abundance of defensive polymorphs within the colonies decreased (Lombardi et al. 2011b).

Polychaete larvae were only encountered in low numbers in the mesocosms, and similar to other pelagic mesocosm experiments (Troedsson et al. 2012, Niehoff et al. 2013), their abundance was not affected by elevated pCO_2. A benthic microcosms study indicated that also the abundance of benthic life stages, i.e. juveniles and adults, was not influenced by CO_2 (Kurihara et al. 2007). A single species study showed that CO_2 induced seawater acidification did also not increase mortality in *Nereis virens* (Widdicombe and Needham 2007). However, in those polychaetes that build calcareous

tubes, the tubes may be weakened due to ocean acidification, likely resulting in reduced survival of the polychaetes and therefore reduced abundances when facing OA (Cigliano et al. 2010, Chan et al. 2012). Accordingly, the polychaete community structure might change in future oceans (Benedetti-Cecchi et al. 2010, Calosi et al. 2013) even though the larvae can tolerate elevated pCO_2.

Appendicularians were rare in the mesocosms at the beginning of the experiment, but increased significantly in abundance during the second bloom phase, indicating that they successfully reproduced. The lacking water movements in the mesocosms as compared to the fjord might have favoured the development of the fragile organisms. Troedsson et al. (2012) just recently studied the response of appendicularians to ocean acidification in a small-scale, 15-day mesocosm study and found that abundances increased significantly at 1000 µatm CO_2 at both, ambient and elevated water temperatures, but only during the phytoplankton bloom. The authors suggest that OA affects fecundity and/or juvenile survival and concluded that appendicularians might play an increasingly important role in future oceans (Troedsson et al. 2012). In our large-scale mesocosm study we were not able to detect significant CO_2 effects on these animals. However, Troedsson et al. (2012) sampled their mesocosms every two days. We were, in contrast, only able to sample once a week for mesozooplankton abundance. As appendicularians seem to be only significantly affected during the chlorophyll *a* maximum (Troedsson et al. 2012), the relatively low temporal resolution of our samplings might not have been sufficient to detect CO_2-related differences in abundance, and, overall, OA effects seem to have at most a minor influence on the total appendicularian community.

In summary, our 33-day mesocosm study on the response of a natural zooplankton community to OA did not reveal significant effects on the community in the water column. Bivalve and gastropod larvae as well as *Pseudocalanus elongatus* nauplii, however, where significantly affected by elevated seawater pCO_2, indicating that long-term exposure would have changed the community structure of high and low pCO_2 mesocosms. It would therefore be of high value to extend the duration of future mesocosm studies. Furthermore, as ocean acidification does not appear in isolation, but in combination with other changing environmental factors such as temperature,

combined effects of future stressors should be included not only in laboratory studies on single species, but also in larger, community-scale studies.

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REFERENCES

Allgaier, M., Riebesell, U., Vogt, M., Thyrhaug, R. and Grossart, H.-P. (2008) Coupling of heterotrophic bacteria to phytoplankton bloom development at different pCO_2 levels: a mesocosm study. *Biogeosciences* **5**, 1007-1022.

Bates, D., Maechler, M., Bolker, B. and Walker, S. (2013) lme4: Linear mixed-effects models using Eigen and S4. R package version 1.0-4. http://CRAN.R-project.org/package=lme4.

Benedetti-Cecchi, L., Iken, K., Konar, B., Cruz-Motta, J., Knowlton, A., Pohle, G., Castelli, A., Tamburello, L., Mead, A., Trott, T., Miloslavich, P., Wong, M., Shirayama, Y., Lardicci, C., Palomo, G. and Maggi, E. (2010) Spatial relationships between polychaete assemblages and environmental variables over broad geographical scales. *PLoS ONE* **5** (9), e12946. doi:10.1371/journal.pone.0012946.

Brussard, C. P. D., Noordeloos, A. A. M., Witte, H., Collenteur, M. C. J., Schulz, K., Ludwig, A. and Riebesell, U. (2013). Arctic microbial community dynamics influenced by elevated CO_2 levels. *Biogeosciences* **10**, 719-731.

Caldeira, K. and Wickett, M. E. (2003) Anthropogenic carbon and ocean pH. *Nature* **425**, 365.

Calosi, P., Rastrick, S. P. S., Lombardi, C., de Guzman, H. J., Davidson, L., Jahnke, M., Giangrande, A., Hardege, J. D., Schulze, A., Spicer, J. I. and Gambi, M.-C. (2013) Adaptation and acclimatization to ocean acidification in marine ectotherms: an *in situ* transplant experiment with polychaetes at a shallow CO_2 vent system. Phil. Trans. R. Soc. B 368, 20120444. doi:10.1098/rstb.2012.0444.

Chan, V. B. S., Li, C., Lane, A. C., Wang, Y., Lu, X., Shih, K., Zhang, T. and Thiyagarajan, V. (2012) CO_2-driven ocean acidification alters and weakens integrity of the calcareous tubes produced by the serpulid tubeworm, *Hydroides elegans*. *PLoS ONE* **7** (8), e42718. doi:10.1371/journal.pone.0042718.

Christen, N., Calosi, P., McNeill, C. L. and Widdicombe, S. (2013) Structural and functional vulnerability to elevated pCO_2 in marine benthic communities. *Mar. Biol*. **160**, 2113-2128.

Cigliano, M., Gambi, M. C., Rodolfo-Metalpa, R., Patti, F. P. and Hall-Spencer, J. M. (2010) Effects of ocean acidification on invertebrate settlement at volcanic CO_2 vents. *Mar. Biol.* **157**, 2489-2502.

Clark, D., Lamare, M. and Barker, M. (2009) Response of sea urchin pluteus larvae (Echinodermata: Echinoidea) to reduced seawater pH: a comparison among a tropical, temperate, and a polar species. *Mar. Biol*. **156**, 1125-1137.

Comeau, S., Gorsky, G., Alliouane, S. and Gattuso, J.-P. (2010) Larvae of the pteropod *Cavolinia inflexa* exposed to aragonite undersaturation are viable but shell-less. *Mar. Biol*. **157**, 2341-2345.

Comeau, S., Alliouane, S. and Gattuso, J.-P. (2012) Effects of ocean acidification on overwintering juvenile Arctic pteropods *Limacina helicina*. *Mar. Ecol. Prog. Ser*. **456**, 279-284.

De Kluijver, A., Soetaert, K., Czerny, J., Schulz, K. G., Boxhammer, T., Riebesell, U. and Middelburg, J. J. (2013) A ^{13}C labelling study on carbon fluxes in Arctic plankton communities under elevated CO_2 levels. *Biogeosciences* **10**, 1425-1440.

Delille, B., Harlay, J., Zondervan, I., Jacquet, S., Chou, L., Wollast, R., Bellerby, R. G. J., Frankignoulle, M., Vieira Borges, A., Riebesell, U. and Gattuso, J.-P. (2005) Response of primary production and calcification to changes of pCO_2 during experimental blooms of the coccolithophorid *Emiliania huxleyi*. *Glob. Biogeochem. Cycle* **19** (2), GB2023, doi:10.1029/2004GB002318.

Dupont, S. and Thorndyke, M. C. (2008) Ocean acidification and its impact on the early life-history stages of marine animals. *CIESM Workshop Monographs* **36**, 89--97.

Dupont, S., Havenhand, J., Thorndyke, W., Peck, L. and Thorndyke, M. (2008) Near-future level of CO_2-driven ocean acidification radically affects larval survival and development in the brittlestar *Ophiothrix fragilis*. *Mar. Ecol. Prog. Ser*. **373**, 285-294.

Ellis, R. P., Bersey, J., Rundle, S. D., Hall-Spencer, J. M. and Spicer, J. I. (2009) Subtle but significant effects of CO_2 acidified seawater on embryos of the intertidal snail, *Littorina obtusata*. *Aquatic Biol*. **5**, 41-48.

Engel, A., Delille, B., Jacquet, S., Riebesell, U., Rochelle-Newall, E., Terbrüggen, A. and Zondervan, I. (2004) Transparent exopolymer particles and dissolved organic carbon production by *Emiliania huxleyi* exposed to different CO_2 concentrations: a mesocosm experiment. *Aquatic Microb. Ecol*. **34**, 93-104.

Engel, A., Schulz, K. G., Riebesell, U., Bellerby, R., Delille, B. and Schartau, M. (2008) Effects of CO_2 on particle size distribution and phytoplankton abundance during a mesocosm bloom experiment (PeECE II). *Biogeosciences* **5**, 509-521.

Feely, R. A., Doney, S. C. and Cooley, S. R. (2009) Ocean acidification. Present conditions and future changes in a high-CO_2 world. *Oceanography* **22** (4), 36-47.

Findlay, H. S., Kendall, M. A., Spicer, J. I., Turley, C. and Widdicombe, S. (2008) Novel microcosm system for investigating the effects of elevated carbon dioxide and temperature on intertidal organisms. *Aquatic Biol.* **3**, 51-62.

Findlay, H. S., Kendall, M. A., Spicer, J. I. and Widdicombe, S. (2009) Future high CO_2 in the intertidal may compromise adult barnacle *Semibalanus balanoides* survival and embryonic development rate. *Mar. Ecol. Prog. Ser.* **389**, 193-202.

Findlay, H. S., Kendall, M. A., Spicer, J. I. and Widdicombe, S. (2010) Relative influences of ocean acidification and temperature on intertidal barnacle post-larvae at the northern edge of their geographic distribution. *Estuar. Coast. Shelf Sci*. **86**, 675-682.

Fitzer, S. C., Caldwell, G. S., Close, A. J., Clare, A. S., Upstill-Goddard, R. C. and Bentley, M. G. (2012) Ocean acidification induces multi-generational decline in copepod naupliar production with possible conflict for reproductive resource allocation. *J. Exp. Mar. Biol. Ecol.* **418-419**, 30-36.

Gonzalez-Bernat, M. J., Lamare, M. and Barker, M. (2013) Effects of reduced seawater pH on fertilization, embryogenesis and larval development in the Antarctic seastar *Odontaster validus*. *Polar Biol*. **36** (2), 235-247.

Grossart, H.-P., Allgaier, M., Passow, U. and Riebesell, U. (2006) Testing the effect of CO_2 concentration on the dynamics of marine heterotrophic bacterioplankton. *Limnol. Oceanogr*. **51** (1), 1-11.

Hall-Spencer, J. M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., Fine, M., Turner, S. M., Rowley, S. J., Tedesco, D. and Buia, M.-C. (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature* **454**, 96-99.

Havenhand, J. N., Buttler, F.-R., Thorndyke, M. C. and Williamson, J. E. (2008) Near-future levels of ocean acidification reduce fertilization success in a sea urchin. *Curr. Biol*. **18** (15), 651-652.

Hildebrandt, N., Niehoff, B. and Sartoris, F.-J. (in press) Long-term effects of elevated CO_2 and temperature on the Arctic calanoid copepods *Calanus glacialis* and *C. hyperboreus*. *Mar. Pollut. Bull.*

Kawaguchi, S., Kurihara, H., King, R., Hale, L., Berli, T., Robinson, J. P., Ishida, A., Wakita, M., Virtue, P., Nicol, S. and Ishimatsu, A. (2011) Will krill fare well under southern ocean acidification? *Biol. Lett*. **7**, 288-291.

Kim, J.-M., Lee, K., Shin, K., Kang, J.-H., Lee, H.-W., Kim, M., Jang, P.-G. and Jang, M.-C. (2006) The effect of seawater CO_2 concentration on growth of a natural

phytoplankton assemblage in a controlled mesocosm experiment. *Limnol. Oceanogr*. **51** (4), 1629-1636.

Kim, J.-M., Lee, K., Shin, K., Yang, E. J., Engel, A., Karl, D. M. and Kim, H.-C. (2011) Shifts in biogenic carbon flow from particulate to dissolved forms under high carbon dioxide and warm ocean conditions. Geophys. Res. Lett. 38, L08612, doi:10.1029/2011GL047346.

Kroeker, K. J., Micheli, F., Gambi, M. C. And Martz, T. R. (2011) Divergent ecosystem responses within a benthic marine community to ocean acidification. *PNAS* **108** (35), 14515-14520.

Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M. and Gattuso, J.-P. (2013) Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Glob. Change Biol*. **19**, 1884-1896.

Kurihara, H., Ishimatsu, A. and Shirayama, Y. (2007) Effects of elevated seawater CO_2 concentration on the meiofauna. *J. Mar. Sci. Technol. Taiwan* **15**, 17-22.

Kurihara, H. and Ishimatsu, A. (2008) Effects of high CO_2 seawater on the copepod (*Acartia tsuensis*) through all life stages and subsequent generations. *Mar. Pollut. Bull*. **56**, 1086-1090.

Kurihara, H., Shimode, S. and Shirayama, Y. (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.

Kurihara, H., Matsui, M., Furukawa, H. and Hayashi, M. (2008) Long-term effects of predicted future seawater CO_2 conditions on the survival and growth of the marine shrimp *Palaemon pacificus*. *J. Exp. Mar. Biol. Ecol*. **367**, 41-46.

Li, W. and Gao, K. (2012) A marine secondary producer respires and feeds more in a high CO_2 ocean. *Mar. Pollut. Bull*. **64**, 699-703.

Lischka, S., Büdenbender, J., Boxhammer, T. and Riebesell, U. (2011) Impact of ocean acidification and elevated temperatures on early juveniles of the polar shelled pteropod *Limacina helicina*: mortality, shell degradation, and shell growth. *Biogeosciences* **8**, 919-932.

Lombardi, C., Rodolfo-Metalpa, R., Cocito, S., Gambi, M. C. and Taylor, P. D. (2011a) Structural and geochemical alterations in the Mg calcite bryozoan *Myriapora truncata* under elevated seawater pCO_2 simulation ocean acidification. *Mar. Ecol.* **32** (2), 211-221.

Lombardi, C., Gambi, M. C., Vasapollo, C., Taylor, P. and Cocito, S. (2011b) Skeletal alterations and polymorphism in a Mediterranean bryozoan at natural CO_2 vents. *Zoomorphology* **130**, 135-145.

Longhurst, A. R. (1985) The structure and evolution of plankton communities. *Prog. Oceanogr*. **15**, 1-35.

Matthews, J. B. L. (1967) On the calanoid copepods of Raunefjorden, western Norway. *Sarsia* **29**, 159-164.

Mayor, D. J., Matthews, C., Cook, K., Zuur, A. F. and Hay, S. (2007) CO_2-induced acidification affects hatching success in *Calanus finmarchicus*. *Mar. Ecol. Prog. Ser*. **350**, 91-97.

Mayor, D. J., Everett, N. R. and Cook, K. B. (2012) End of century ocean warming and acidification effects on reproductive success in a temperate marine copepod. *J. Plankton Res*. **34** (3), 258-262.

McConville, K., Halsband, C., Fileman, E. S., Somerfield, P. J. and Findlay, H. S. (2013) Effects of elevated CO_2 on the reproduction of two calanoid copepods. *Mar. Pollut. Bull.* http://dx.doi.org/10.1016/j.marpolbul.2013.02.010.

Meinshausen, M., Smith, S. J., Calvin, K., Daniel, J. S., Kainuma, M. L. T., Lamarque, J.-F., Matsumoto, K., Montzka, S. A., Raper, S. C. B., Riahi, K., Thomson, A., Velders, G. J. M., van Vuuren, D. P. P. (2011) The RCP greenhouse gas concentrations and their extensions from 1765 to 2300. *Clim. Change* **109**, 213-241.

Melatunan, S., Calosi, P., Rundle, S. D., Widdicombe, S. and Moody, A. J. (2013) Effects of ocean acidification and elevated temperature on shell plasticity and its energetic basis in an intertidal gastropod. *Mar. Ecol. Prog. Ser*. **472**, 155-168.

Melzner, F., Stange, P., Trübenbach, K., Thomsen, J., Casties, I., Panknin, U., Gorb, S. N. and Gutowska, M. A. (2011) Food supply and seawater pCO_2 impact calcification and internal shell dissolution in the blue mussel *Mytilus edulis*. *PLoS ONE* **6** (9), e24223. doi:10.1371/journal.pone.0024223.

Michaelidis, B., Ouzounis, C., Paleras, A. and Pörtner, H. O. (2005) Effects of long-term moderate hypercapnia on acid-base balance and growth rate in marine mussels *Mytilus galloprovincialis*. *Mar. Ecol. Prog. Ser.* **293**, 109-118.

Moss, R. H., Edmonds, J. A., Hibbard, K. A., Manning, M. R., Rose, S. K., van Vuuren, D. P., Carter, T. R., Emori, S., Kainuma, M., Kram, T., Meehl, G. A., Mitchell, J. F. B., Nakicenovic, N., Riahi, K., Smith, S. J., Stouffer, R. J., Thomson, A. M., Weyant, J. P. and Wilbanks, T. J. (2010) The next generation of scenarios for climate change research and assessment. *Nature* **463**, 747-756.

Munday, P. L., Dixson, D. L., Donelson, J. M., Jones, G. P., Pratchett, M. S., Devitsina, G. V. and Døving, K. B. (2009) Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *PNAS* **106** (6), 1848-1852.

Munday, P. L., Dixson, D. L., McCormick, M. I., Meekan, M., Ferrari, M. C. O. and Chivers, D. P. (2010) Replenishment of fish populations is threatened by ocean acidification. *PNAS* **107** (29), 12930-12934.

Nejstgaard, J. C., Frischer, M. E., Verity, P. G., Anderson, J. T., Jacobsen, A., Zirbel, M. J., Larsen, A., Martínez-Martínez, J., Sazhin, A. F., Walters, T., Bronk, D. A., Whipple, S. J., Borrett, S. R., Patten, B. C. and Long, J. D. (2006) Plankton

development and trophic transfer in seawater enclosures with nutrients and *Phaeocystis pouchetii* added. *Mar. Ecol. Prog. Ser.* **321**, 99-121.

Nelson, W. G. (1982) Experimental studies of oil pollution on the rocky intertidal community of a Norwegian fjord. *J. Exp. Mar. Biol. Ecol.* **65**, 121-138.

Niehoff, B. and Hirche, H.-J. (2000) The reproduction of *Calanus finmarchicus* in the Norwegian Sea in spring. *Sarsia* **85**, 15-22.

Niehoff, B., Klenke, U., Hirche, H.-J., Irigoien, X., Head, R. and Harris, R. (1999) A high frequency time series at Weathership M, Norwegian Sea, during the 1997 spring bloom: the reproductive biology of *C. finmarchicus*. *Mar. Ecol. Prog. Ser.* **176**, 81-92.

Niehoff, B., Schmithüsen, T., Knüppel, N., Daase, M., Czerny, J. and Boxhammer, T. (2013) Mesozooplankton community development at elevated CO_2 concentrations: results from a mesocosm experiment in an Arctic fjord. *Biogeosciences* **10**, 1391-1406.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Henry, M., Stevens, H. And Wagner, H. (2013) vegan: Community Ecology Package. R package version 2.0-9. http://CRAN.R-project.org/package=vegan.

Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., Key, R. M., Lindsay, K., Maier-Reimer, E., Matear, R., Monfray, P., Mouchet, A., Najjar, R. G., Plattner, G.-K., Rodgers, K. B., Sabine, C. L., Sarmiento, J. L., Schlitzer, R., Slater, R. D., Totterdell, I. J., Weirig, M.-F., Yamanaka, Y. and Yool, A. (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **437**, 681-686.

Pansch, C., Nasrolahi, A., Appelhans, Y. S. and Wahl, M. (2012) Impacts of ocean warming and acidification on the larval development of the barnacle *Amphibalanus improvisus*. *J. Exp. Mar. Biol. Ecol.* **420-421**, 48-55.

Pedersen, S. A., Hansen, B. H., Altin, D. and Olsen, A. J. (2013) Chronic exposure of the North Atlantic copepod *C. finmarchicus* (Gunnerus, 1770) to CO_2-acidified seawater; effects on survival, growth and development. *Biogeosciences Discuss.* **10**, 5273-5300.

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Renz, J., Mengedoht, D. and Hirche, H.-J. (2008) Reproduction, growth and secondary production of *P. elongatus* Boeck (Copepoda, Calanoida) in the southern North Sea. *J. Plankton Res.* **30** (5), 511-528.

Riebesell, U., Bellerby, R. G. J., Grossart, H.-P. and Thingstad, F. (2008) Mesocosm CO_2 perturbation studies: from organism to community level. *Biogeosciences* **5**, 1157-1164.

Riebesell, U., Lee, K. and Neijstgaard, J. C. (2010) Pelagic mesocosms. In: Riebesell, U., Fabry, V. J., Hansson, L. and Gattuso, J.-P. (eds.) *Guide to best practices for ocean acidification research and data reporting.* Publications Office of the European Union, Luxembourg, pp. 95-112.

Riebesell, U., Czerny, J., von Bröckel, K., Boxhammer, T., Büdenbender, J., Deckelnick, M., Fischer, M., Hoffmann, D., Krug, S. A., Lentz, U., Ludwig, A., Muche, R. and Schulz, K. G. (2013) Technical note: A mobile sea-going mesocosm system - new opportunities for ocean acidification research. *Biogeosciences* **10**, 1835-1847.

Ries, J. B., Cohen, A. L. and McCorkle, D. C. (2009) Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. *Geology* **37**, 1131-1134.

Rodolfo-Metalpa, R., Lombardi, C., Cocito, S., Hall-Spencer, J. M. and Gambi, M. C. (2010) Effects of ocean acidification and high temperatures on the bryozoan *Myriapora truncata* at natural CO_2 vents. *Mar. Ecol*. **31** (3), 447-456.

Rosa, R. and Seibel, B. A. (2008) Synergistic effects of climate-related variables suggest future physiological impairment in a top oceanic predator. *PNAS* **105** (52), 20776-20780.

Rossoll, D., Bermúdez, R., Hauss, H., Schulz, K. G., Riebesell, U., Sommer, U. and Winder, M. (2012) Ocean acidification-induced food quality deterioration constrains trophic transfer. *PLoS ONE* **7** (4), e34737. doi:10.1371/journal.pone.0034737.

Rossoll, D., Sommer, U. and Winder, M. (2013) Community interactions dampen acidification effects in a coastal plankton system. *Mar. Ecol. Prog. Ser.* **486**, 37-46.

Saba, G. K., Schofield, O., Torres, J. J., Ombres, E. H. and Steinberg, D. K. (2012) Increased feeding and nutrient excretion of adult Antarctic krill, *Euphausia superba*, exposed to enhanced carbon dioxide (CO_2). *PLoS ONE* **7** (12), e52224. doi:10.1371/journal.pone.0052224.

Sheppard Brennand, H., Soars, N., Dworjanyn, S. A., Davis, A. R. and Byrne, M. (2010) Impact of ocean warming and ocean acidification on larval development and calcification in the sea urchin *Tripneustes gratilla*. *PLoS ONE* **5** (6), e11372. doi:10.1371/journal.pone.0011372

Stumpp, M., Wren, J., Melzner, F., Thorndyke, M. C. and Dupont, S. T. (2011) CO_2 induced seawater acidification impacts sea urchin larval development I: Elevated metabolic rates decrease scope for growth and induce developmental delay. *Comp. Biochem. Physiol. A* **160**, 331-340.

Stumpp, M., Hu, M., Casties, I., Saborowski, R., Blweich, R., Melzner, F. and Dupont, S. (2013) Digestion in sea urchin larvae impaired under ocean acidification. *Nat. Clim. Change*. doi:10.1038/nclimate2018.

Suffrian, K., Simonelli, P., Nejstgaard, J. C., Putzeys, S., Carotenuto, Y. and Antia, A. N. (2008) Microzooplankton grazing and phytoplankton growth in marine mesocosms with increased CO_2 levels. *Biogeosciences* **5**, 1145-1156.

Suwa, R., Nojiri, Y., Ono, T. and Shirayama, Y. (2013) Effects of low pCO_2 conditions on sea urchin larval size. *Mar. Ecol*. doi:10.1111/maec.12044.

Talmage, S. C. and Gobler, C. J. (2010) Effects of past, present, and future ocean carbon dioxide concentrations on the growth and survival of larval shellfish. *PNAS* **107** (40), 17246-17251.

Troedsson, C., Bouquet, J.-M., Lobon, C. M., Novac, A., Neijstgaard, J. C., Dupont, S., Bosak, S., Jakobsen, H. H., Romanova, N., Pankoke, L. M., Isla, A., Dutz, J., Sazhin, A. F. and Thompson, E. M. (2012) Effects of ocean acidification, temperature and nutrient regimes on the appendicularian *Oikopleura dioica*: a mesocosm study. *Mar. Biol*. **160**, 2175-2187.

Tunberg, B. (1981) Two bivalve communities in a shallow and sandy bottom in Raunefjorden, western Norway. *Sarsia* **66** (4), 257-266.

Tunberg, B. (1982) Quantitative distribution of the macrofauna in a shallow, sandy bottom in Raunefjorden, western Norway. *Sarsia* **67** (3), 201-210.

Urabe, J., Togari, J. and Elser, J. J. (2003) Stoichiometric impacts of increased carbon dioxide on a planktonic herbivore. *Glob. Change Biol*. **9**, 818-825.

Urabe, J. and Waki, N. (2009) Mitigation of adverse effects of rising CO_2 on a planktonic herbivore by mixed algal diets. *Glob. Change Biol*. **15**, 523-531.

Vargas, C. A., de la Hoz, M., Aguilera, V., San Martin, V., Manríquez, P. H., Navarro, J. M., Torres, R., Lardies, M. A. and Lagos, N. A. (2013) CO_2-driven ocean acidification reduces larval feeding efficiency and change food selectivity in the mollusc *Concholepas concholepas*. *J. Plankton Res*. 35 (5), 1059-1068.

Vestheim, H., Langford, K. and Hylland, K. (2012) Lack of response in a marine pelagic community to short-term oil and contaminant exposure. *J. Exp. Mar. Biol. Ecol*. **416-417**, 110-114.

Watson, S.-A., Southgate, P. C., Tyler, P. A. and Peck, L. S. (2009) Early larval development of the Sydney rock oyster *Saccostrea glomerata* under near-future predictions of CO_2-driven ocean acidification. *J Shellfish Res*. **28** (3), 431-437.

Weydmann, A., Søreide, J. E., Kwasniewski, S. and Widdicombe, S. (2012) Influence of CO_2-induced acidification on the reproduction of a key Arctic copepod *Calanus glacialis*. *J. Exp. Mar. Biol. Ecol*. **428**, 39-42.

Widdicombe, S. and Needham, H. R. (2007) Impact of CO_2-induced seawater acidification on the burrowing activity of *Nereis virens* and sediment nutrient flux. *Mar. Ecol. Prog. Ser*. **341**, 1111-122.

Widdicombe, S., Dupont, S. and Thorndyke, M. (2010) Laboratory experiments and benthic mesocosm studies. In: Riebesell, U., Fabry, V. J., Hansson, L. and

Gattuso, J.-P. (eds.) *Guide to best practices for ocean acidification research and data reporting*. Publications Office of the European Union, Luxembourg, pp. 113-122.

Wittmann, A. C. and Pörtner, H.-O. (2013) Sensitivities of extant animal taxa to ocean acidification. *Nat. Clim. Change* **3**, 995–1001.

Zhang, D., Li, S., Wang, G. and Guo, D. (2011) Impacts of CO_2-driven seawater acidification on survival, egg production rate and hatching success of four marine copepods. *Acta Oceanol. Sin.* **30** (6), 86-94.

Zervoudaki, S., Frangoulis, C., Giannoudi, L. and Krasakopoulou, E. (2014) Effects of low pH and raised temperature on egg production, hatching and metabolic rates of a Mediterranean copepod species (*Acartia clausi*) under oligotrophic conditions. *Mediterr. Mar. Sci.* **15** (1), 74-83.

SUPPLEMENTARY MATERIAL

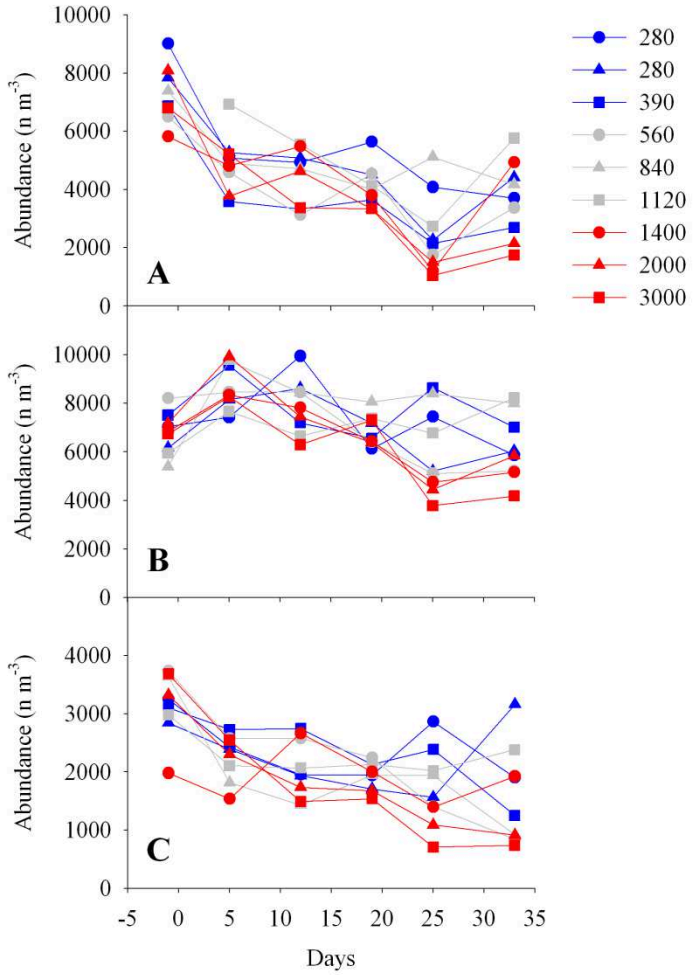

Fig. S1 Total mesozooplankton abundance in the water column of the nine mesocosms: (A) copepod nauplii, (B) copepodites and adult copepods and (C) non-copepod mesozooplankton. Numbers in the legend present initial CO_2 concentrations (µatm) after the CO_2 manipulation was completed.

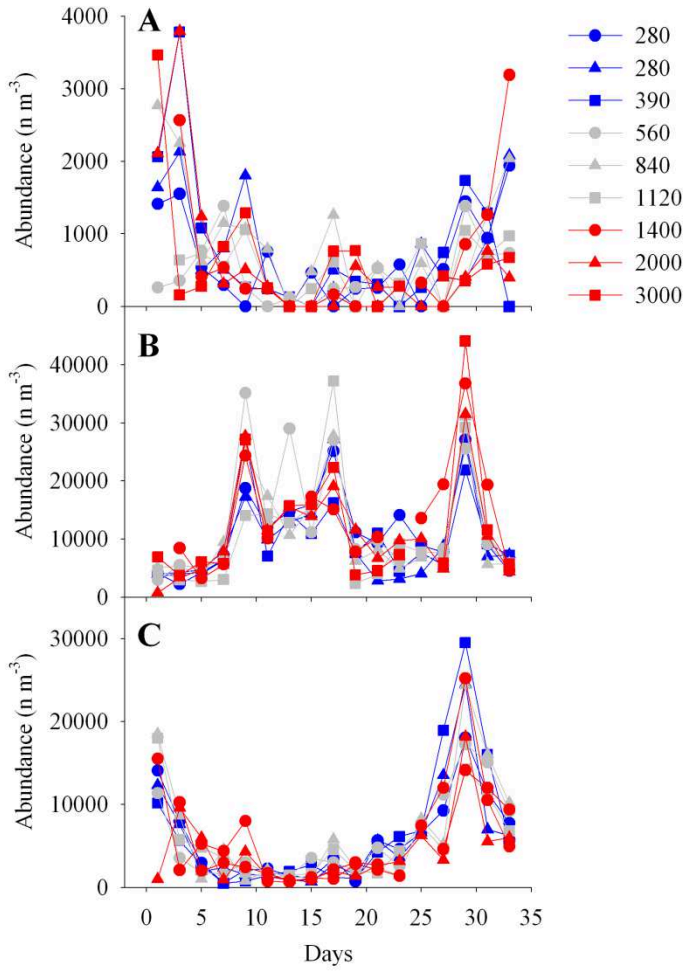

Fig. S2 Total mesozooplankton abundance in the sediment traps of the nine mesocosms: (A) copepod nauplii, (B) copepodites and adult copepods and (C) non-copepod mesozooplankton. Numbers in the legend present initial CO_2 concentrations (µatm) after the CO_2 manipulation was completed.

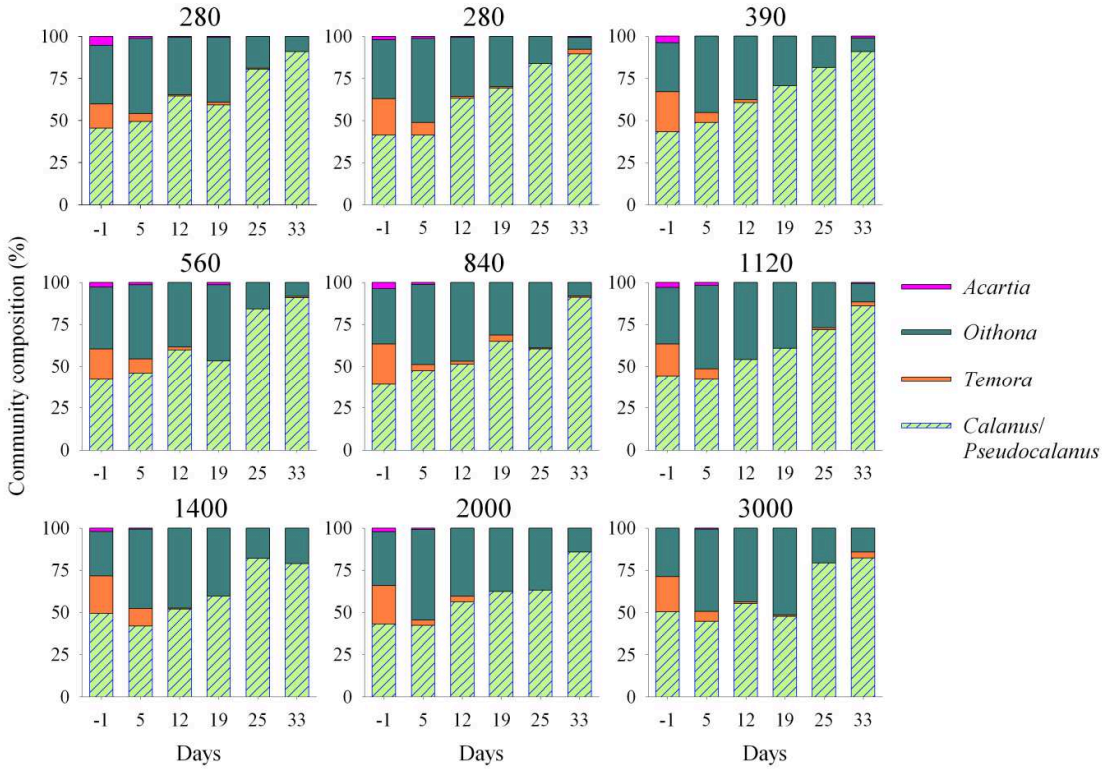

Fig. S3 Copepod community composition (nauplii) (%) in the water column of the nine mesocosms. Numbers on top of the panels present the initial CO_2 concentration in each mesocosm after the CO_2 manipulation was completed.

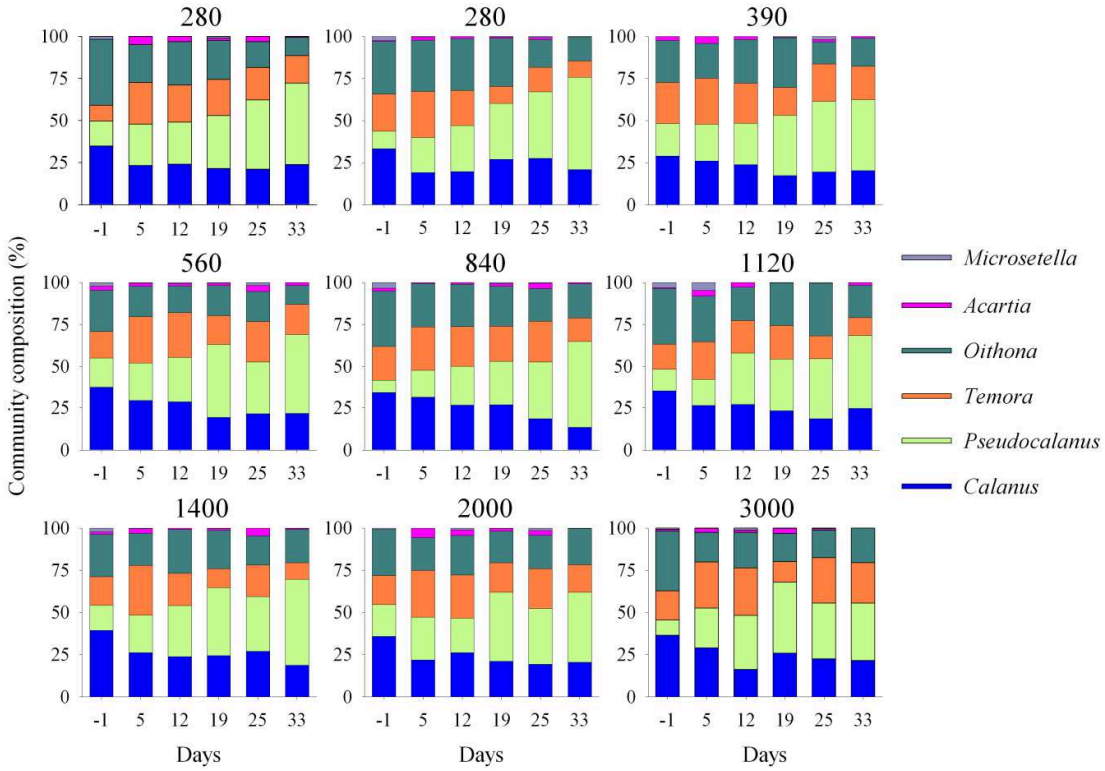

Fig. S4 Copepod community composition (copepodites and adults) (%) in the water column of the nine mesocosms. Numbers on top of the panels present the initial CO_2 concentration in each mesocosm after the CO_2 manipulation was completed.

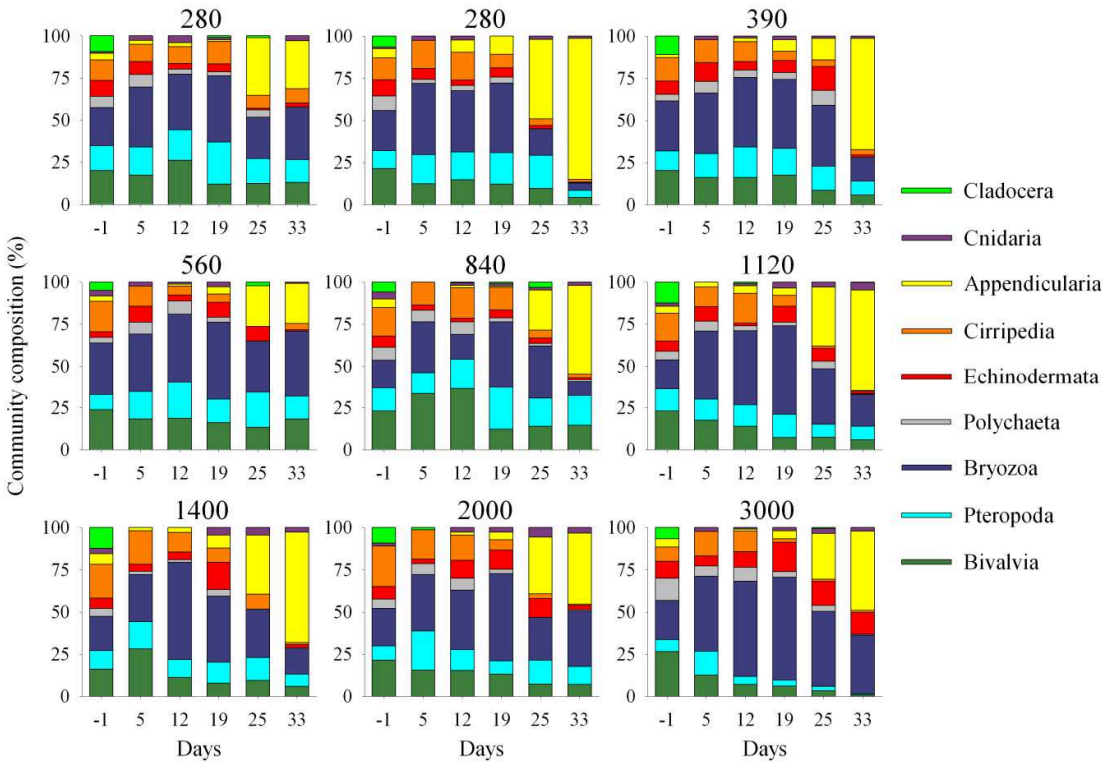

Fig. S5 Mesozooplankton community composition (excluding copepods) (%) in the water column of the nine mesocosms. Numbers on top of the panels present the initial CO_2 concentration in each mesocosm after the CO_2 manipulation was completed.

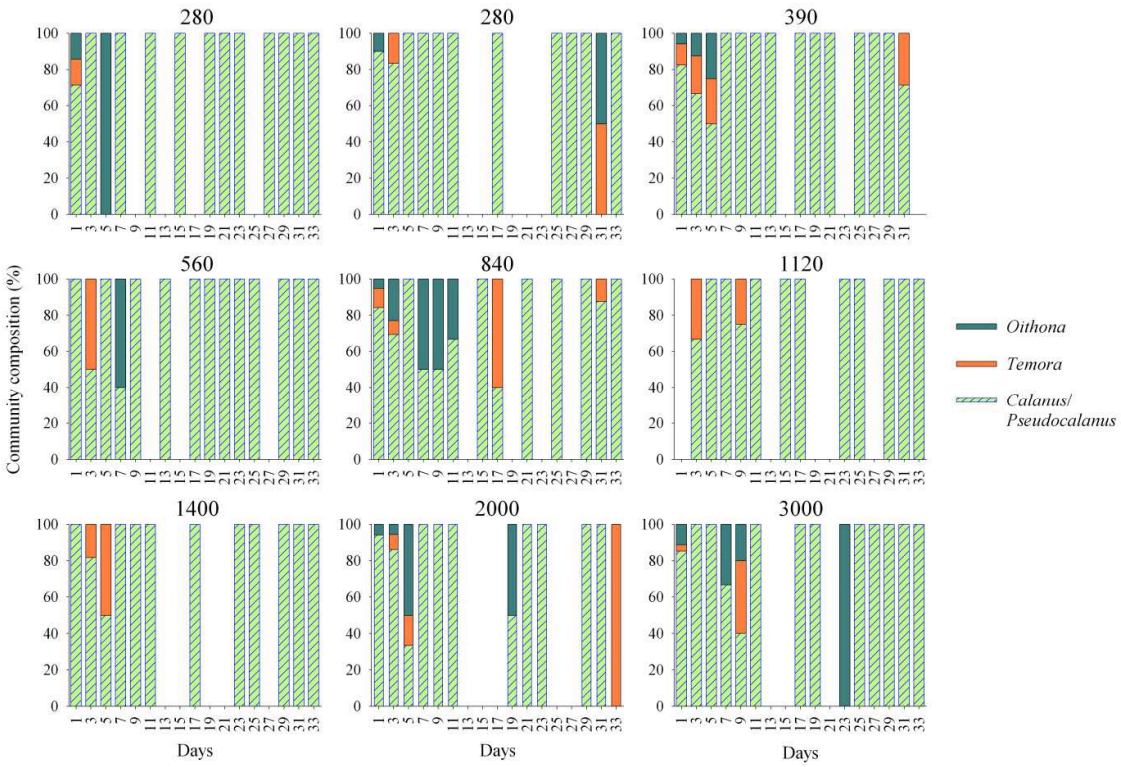

Fig. S6 Copepod community composition (nauplii) (%) in the sediment traps of the nine mesocosms. Numbers on top of the panels present the initial CO_2 concentration in each mesocosm after the CO_2 manipulation was completed. On days without data, no nauplii were present in the samples. In M3 (1120 µatm), no data on nauplii are available for t_{-1}.

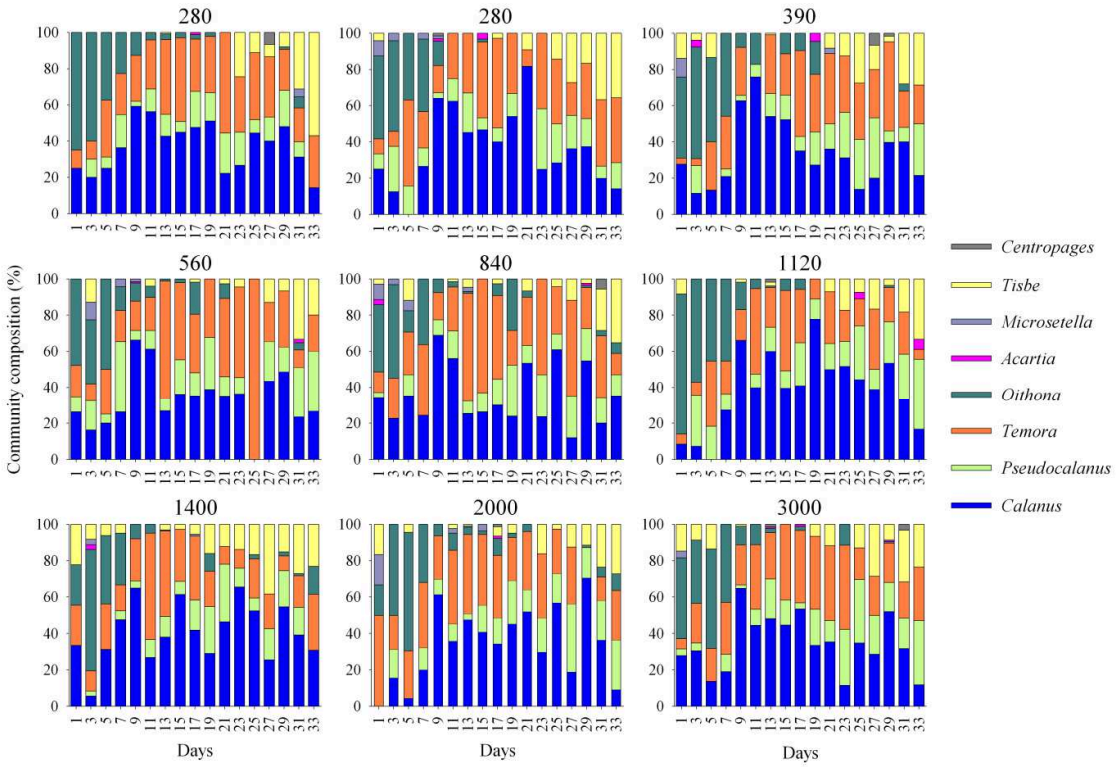

Fig. S7 Copepod community composition (copepodites and adults) (%) in the sediment traps of the nine mesocosms. Numbers on top of the panels present the initial CO_2 concentration in each mesocosm after the CO_2 manipulation was completed.

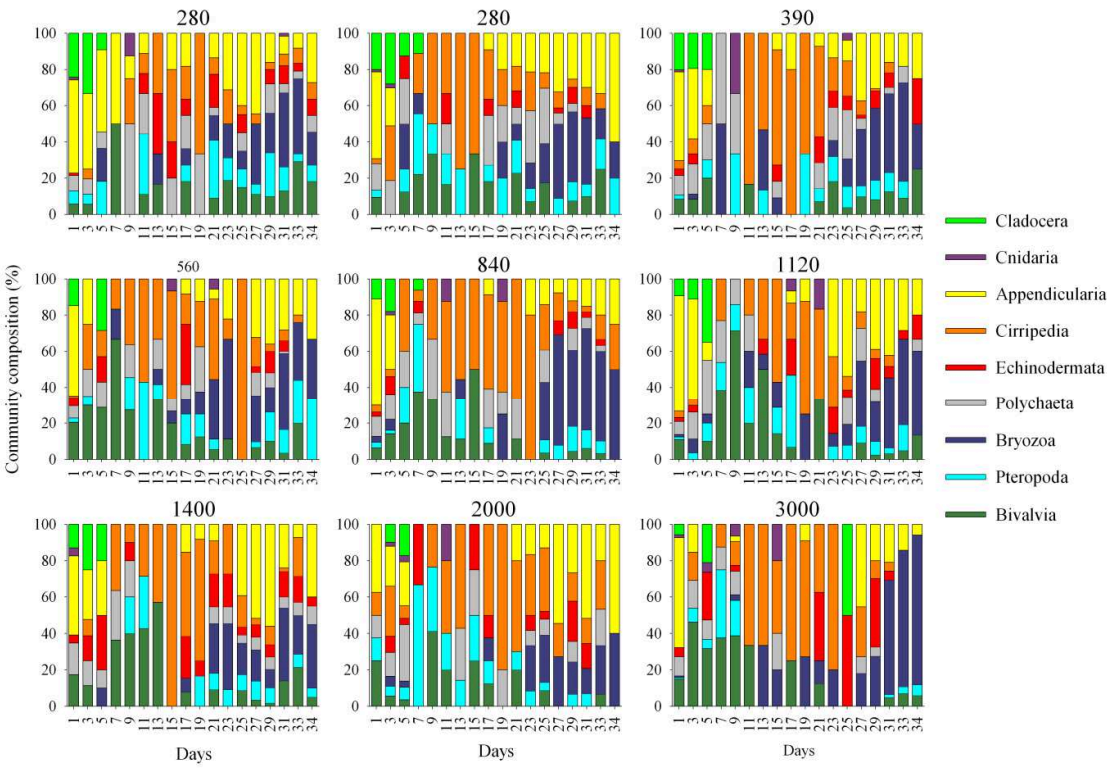

Fig. S8 Mesozooplankton community composition (excluding copepods) (%) in the sediment traps of the nine mesocosms. Numbers on top of the panels present the initial CO_2 concentration in each mesocosm after the CO_2 manipulation was completed.

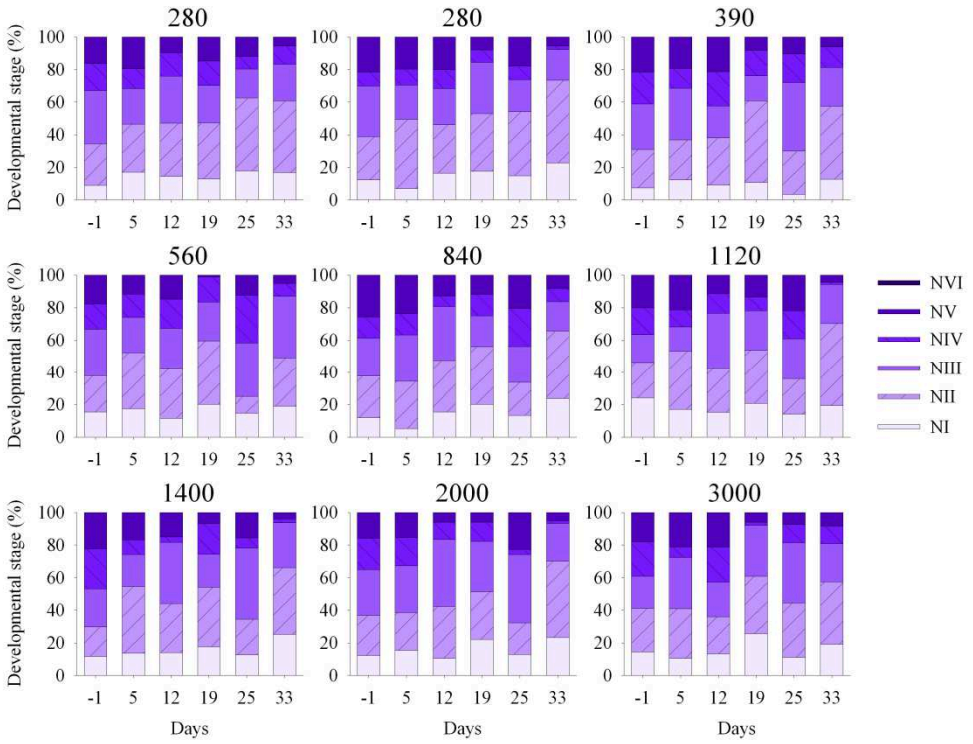

Fig. S9 Developmental stage distribution of *Calanus finmarchicus*/*Pseudocalanus elongatus* nauplii in the water column of the nine mesocosms. Numbers on top of the panels present the initial CO_2 concentration in each mesocosm after the CO_2 manipulation was completed. N = nauplius stage.

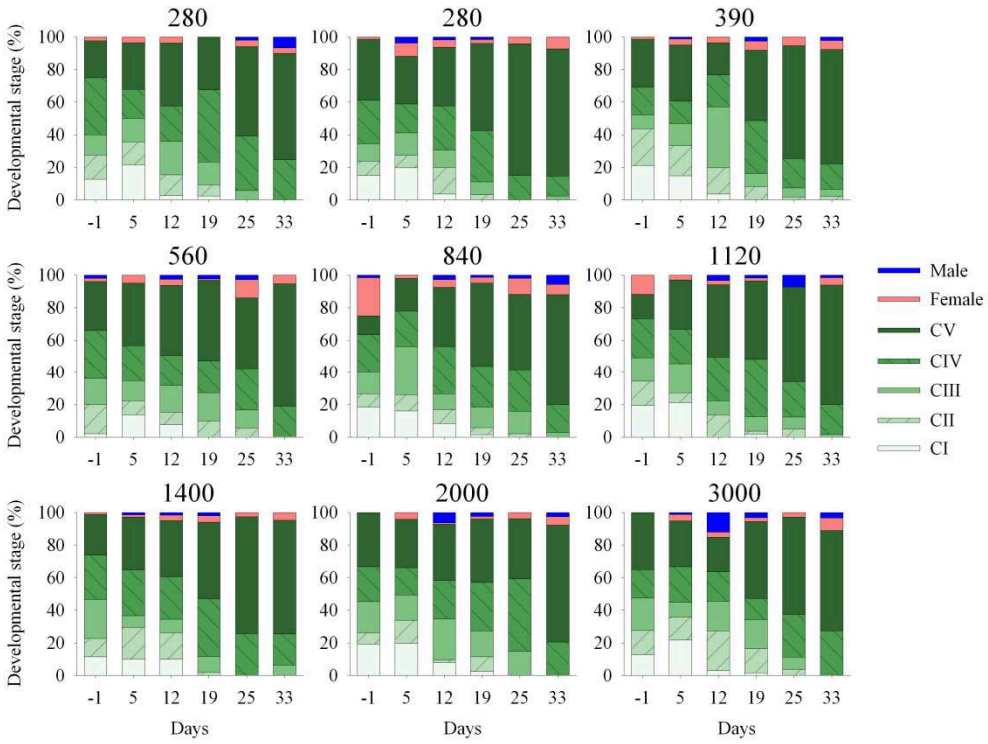

Fig. S10 Developmental stage distribution of *Calanus finmarchicus* copepodites and adults in the water column of the nine mesocosms. Numbers on top of the panels present the initial CO_2 concentration in each mesocosm after the CO_2 manipulation was completed. C = copepodite stage.

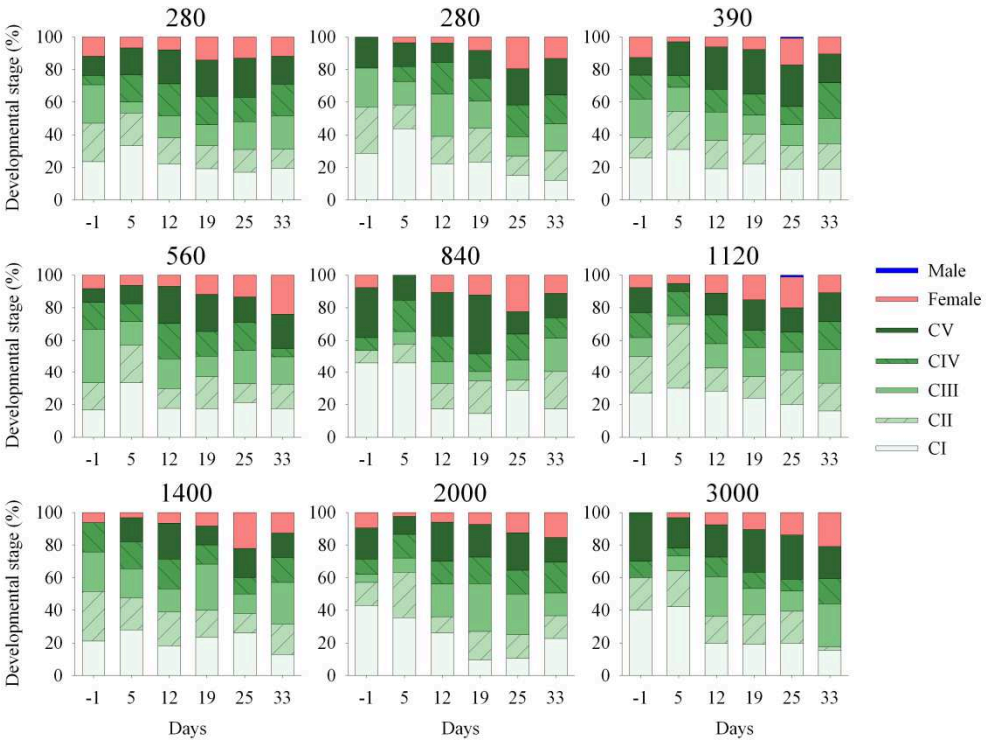

Fig. S11 Developmental stage distribution of *Pseudocalanus elongatus* copepodites and adults in the water column of the nine mesocosms. Numbers on top of the panels present the initial CO_2 concentration in each mesocosm after the CO_2 manipulation was completed. C = copepodite stage.

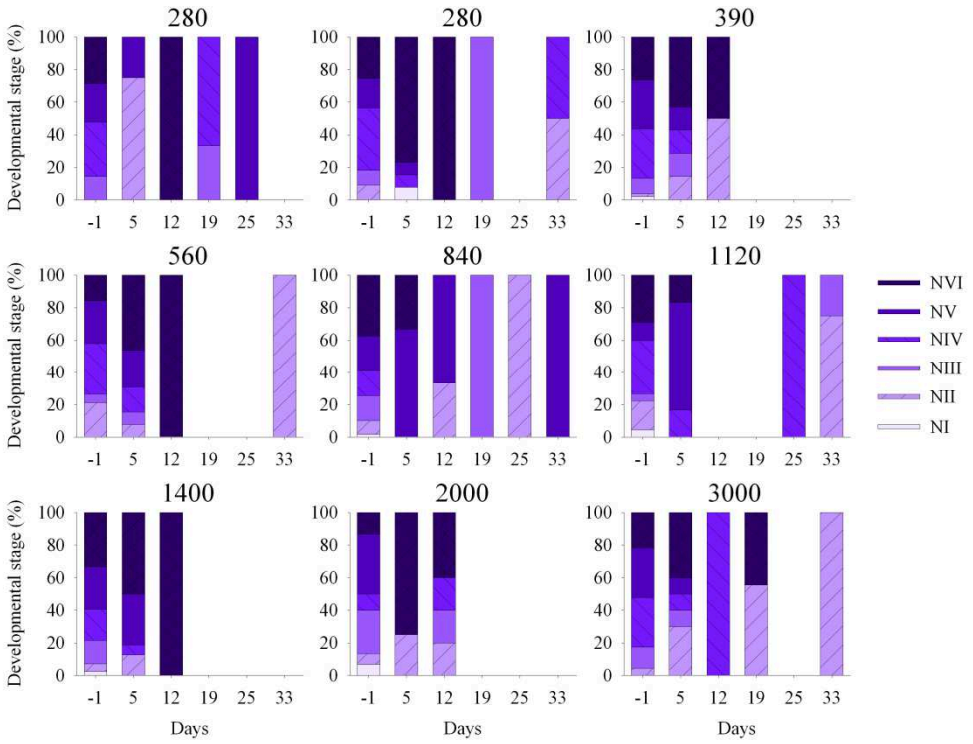

Fig. S12 Developmental stage distribution of *Temora longicornis* nauplii in the water column of the nine mesocosms. Numbers on top of the panels present the initial CO_2 concentration in each mesocosm after the CO_2 manipulation was completed. N = nauplius stage. On days without data, no nauplii were present in the samples.

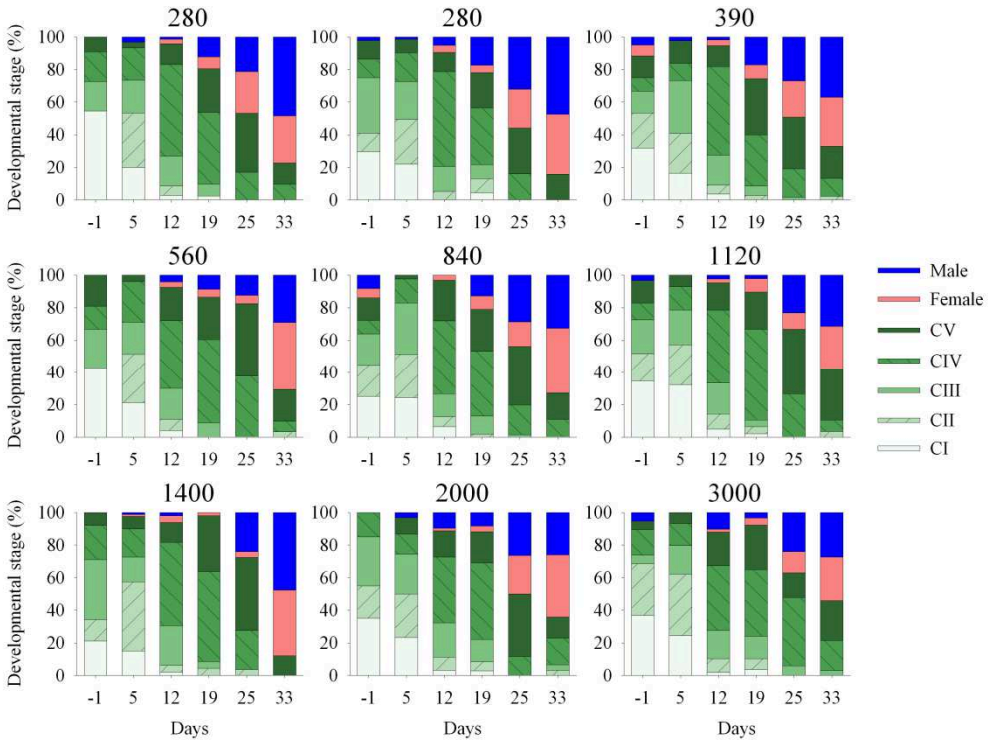

Fig. S13 Developmental stage distribution of *Temora longicornis* copepodites and adults in the water column of the nine mesocosms. Numbers on top of the panels present the initial CO_2 concentration in each mesocosm after the CO_2 manipulation was completed. C = copepodite stage.

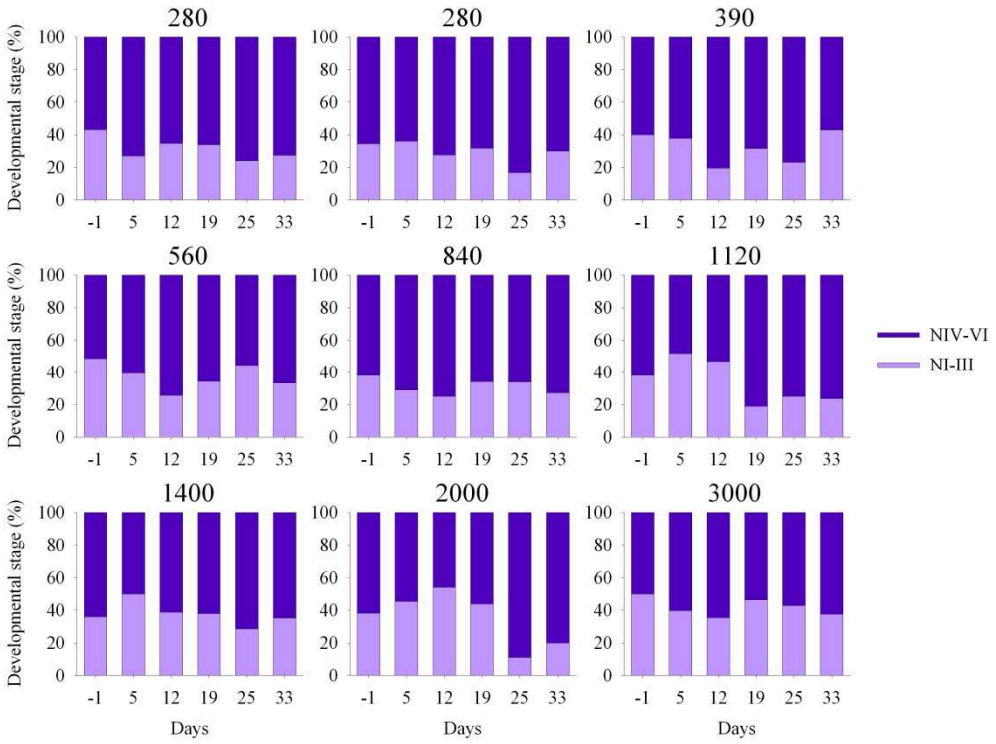

Fig. S14 Developmental stage distribution of *Oithona similis* nauplii in the water column of the nine mesocosms. Numbers on top of the panels present the initial CO_2 concentration in each mesocosm after the CO_2 manipulation was completed. N = nauplius stage.

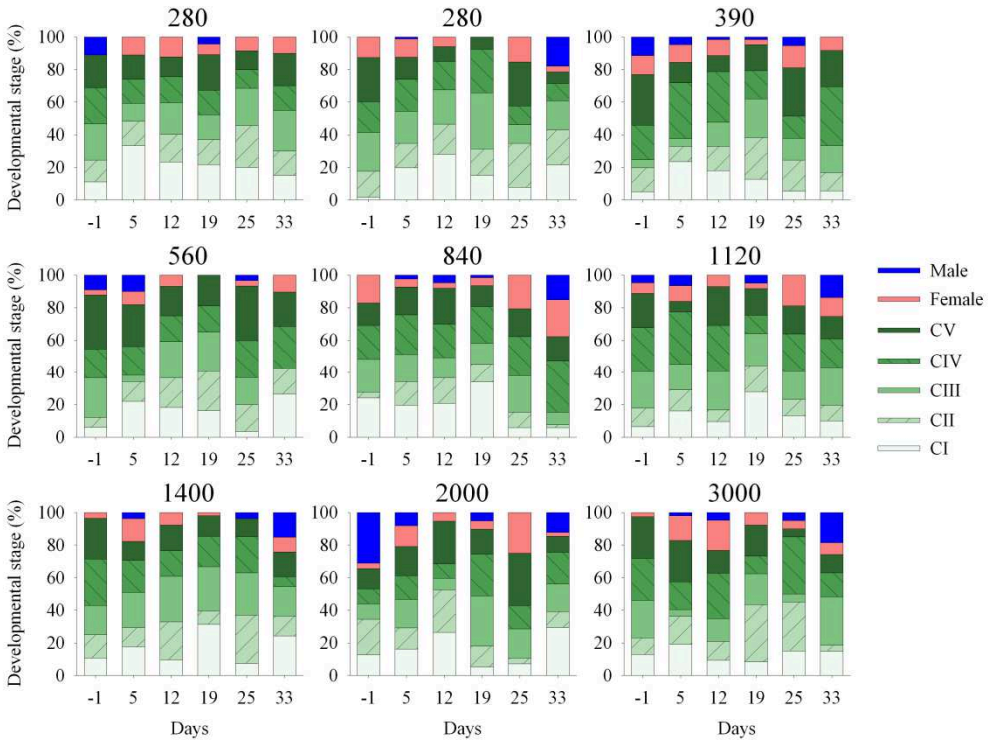

Fig. S15 Developmental stage distribution of *Oithona similis* copepodites and adults in the water column of the nine mesocosms. Numbers on top of the panels present the initial CO_2 concentration in each mesocosm after the CO_2 manipulation was completed. C = copepodite stage.

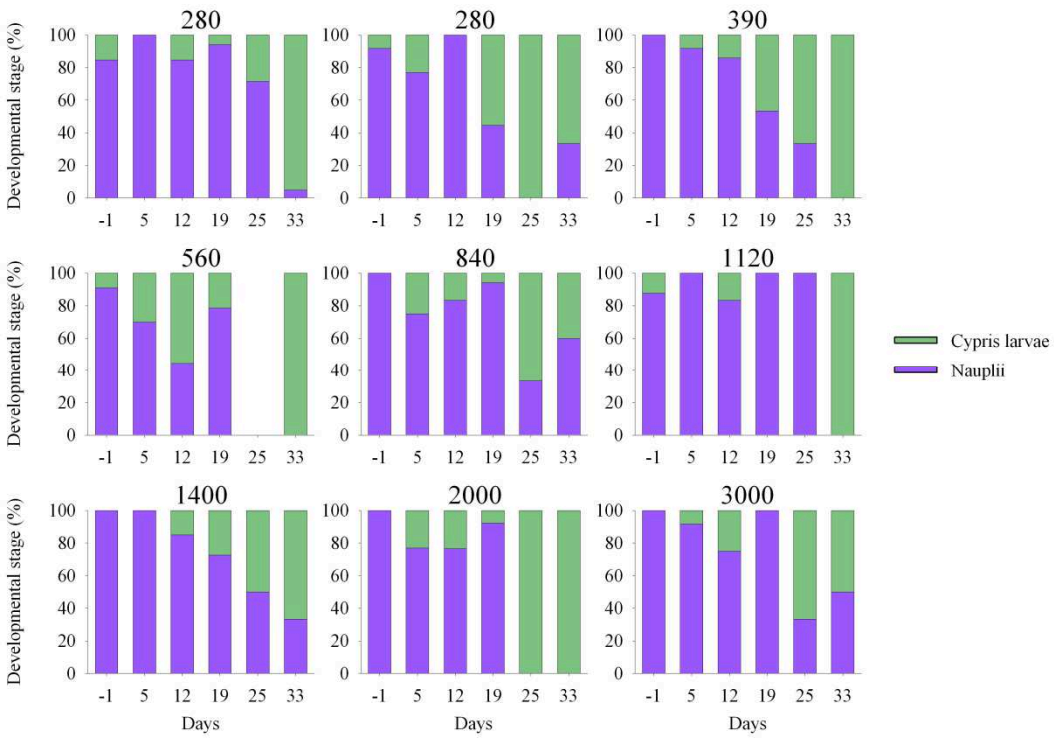

Fig. S16 Developmental stage distribution of cirripedia nauplii and cypris larvae in the water column of the nine mesocosms. Numbers on top of the panels present the initial CO_2 concentration in each mesocosm after the CO_2 manipulation was completed. On days without data, no cirripeds were present in the samples.

4 RESULTS AND SYNOPTIC DISCUSSION

Due to anthropogenic activities, the world's oceans nowadays are facing rapid physical and chemical changes. Ocean acidification, i.e. a decreasing seawater pH due to the uptake of man-made CO_2, may pose a threat to many marine species. The present study investigates how three key species of the Arctic pelagic ecosystem, the herbivorous calanoid copepods *Calanus glacialis*, *C. hyperboreus* and *C. finmarchicus*, respond to the direct and indirect effects of anthropogenic ocean acidification and whether an interaction of ocean acidification and ocean warming will affect them.

4.1 Facing ocean acidification: the response of Arctic *Calanus* spp.

4.1.1 Direct effects

Sub-adult and adult stages of the Arctic *Calanus* spp. are known to be able to survive high seawater CO_2 concentrations of up to 8000 µatm during short-term exposures < 10 days (Marshall et al. 1935, Mayor et al. 2007, Weydmann et al. 2012, Lewis et al. 2013). The present study demonstrates that these copepods can also tolerate a pCO_2 of 3000 µatm (pH 7.2), which is well beyond the worst-case scenario predicted for the year 2300 (Caldeira and Wickett 2003), for an extended period of up to four months (**Publication I-III**). As OA, however, can induce sublethal effects that might alter the fitness of the animals (e.g. Kurihara et al. 2008, Saba et al. 2012, Stumpp et al. 2013) and consequently their population development, which can finally affect whole ecosystems, we need to look beyond adult mortality as an endpoint (Mayor et al. 2007).

As an adaptation to the strong seasonality in food availability in high latitude waters (Lalli and Parsons 1997), the Arctic *Calanus* spp. exhibit plastic multi-year life cycles with an active phase in spring and summer and a resting phase in fall and winter (reviewed e.g. by Falk-Petersen et al. 2009). Energy reserves, which are built up during activity, determine the maximum diapause duration (e.g. Saumweber and Durbin 2006) and, in *C. hyperboreus* females, also the maximum reproductive output (e.g. Hirche and Niehoff 1996). During this study, the effects of elevated seawater pCO_2 on both active and diapausing copepods were investigated in order to test if OA induces stress to the animals, which may alter the available amount of energy and subsequently processes such as feeding, growth and gonad maturation.

4.1.1.1 Active copepods

Our laboratory experiments on *C. finmarchicus* and *C. glacialis* CV indicate that the energy budget of the copepods is not directly affected by OA during activity. As expected, the mass-specific respiration rates, which are a measure of metabolic activity, were higher in small as compared to large copepod species and in active vs. diapausing individuals (Fig. 4.1). Elevated pCO_2 did not significantly affect respiration (**Publication I**, Fig. 4.1) and therefore the energetic demand of the *Calanus* spp. under

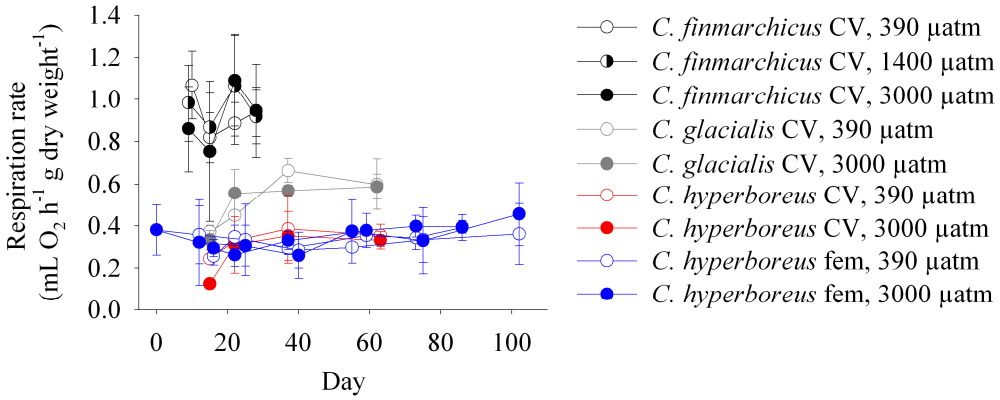

Fig. 4.1 Mass-specific respiration rates of active *Calanus finmarchicus* CV (at 10 °C), active *C. glacialis* CV (at 0 °C) and diapausing *C. hyperboreus* CV and females (at 0 °C) at different CO_2 concentrations.

OA conditions. Accordingly, ingestion was not altered, and body mass as a measure of assimilation and usage of energy reserves did not change in relation to seawater pCO_2 (**Publication II**). Another parameter which has been discussed as an index for secondary production (e.g. McLaren and Leonard 1995) and thus as an index for nutritional status is egg production, as *Calanus* females do not grow in size but invest in gonad development and spawning. During the mesocosm study in Bergen, Norway, we measured egg production rates (EPR) in *C. finmarchicus* females, which are highly correlated to the availability and quality of food algae (e.g. Niehoff 2004). No information can be given on individual clutch size and the percentage of spawning individuals, as up to five females were generally pooled for the experiments. EPR were, however, low in all mesocosms throughout the study, ranging on average between 0 and 17 eggs female^{-1} day^{-1} (Fig. 4.2). Such low EPR are typical for a post-bloom period (e.g. Niehoff et al. 1999, Swalethorp et al. 2011), which was encountered in the Raunefjord at the time of the study (K. G. Schulz (GEOMAR), unpubl. data). During bloom events,

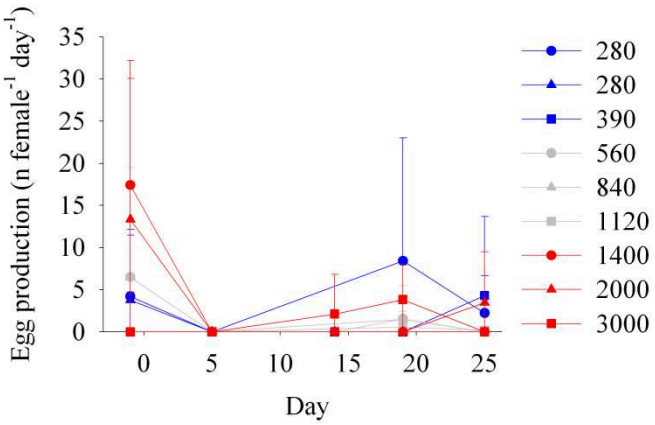

Fig. 4.2 Egg production of *Calanus finmarchicus* sampled during the SOPRAN mesocosm experiment. Numbers in the legend present initial CO_2 concentrations (µatm) in the mesocosms. Data on day -1 represent control values, which were measured before CO_2 manipulation. n ranged between 1 and 30 females (ø 11 ± 6 females).

in contrast, EPR are considerably higher (e.g. Melle and Skjoldal 1998, Niehoff et al. 1999) and may exceed 80 eggs female^{-1} day^{-1} (e.g. Plourde and Runge 1993). No significant differences in EPR were found among copepods from different mesocosms, however, as the reproductive period was already at its end, no reasonable conclusions on the effects of OA on egg production of *C. finmarchicus* can be drawn from our study.

Our findings that active *Calanus* spp. are tolerant to OA are similar to those recently published. Neither in *C. finmarchicus* nor in *C. glacialis* females incubated at elevated seawater pCO_2 for five to nine days, EPR were negatively affected, not even at very high CO_2 concentrations of ~8000 µatm (Mayor et al. 2007, Weydmann et al. 2012). In *C. finmarchicus,* which were incubated from eggs to adults at 1080, 2080 and 3080 µatm for two subsequent generations, survival and developmental times of nauplii and copepodite stages were generally not impaired when compared to copepods from a control treatment (380 µatm), despite some significant, but non-consistent effects on the developmental time in particular life stages (Gustavson 2013, Håkedal 2013). Only at a pCO_2 of ~8000 µatm, egg hatching was significantly delayed in *C. glacialis* and *C. finmarchicus* as compared to control conditions (Mayor et al. 2007, Weydmann et al. 2012). In accordance, a full life cycle study with *C. finmarchicus* incubated at very high CO_2 concentrations of 3300 - 9700 µatm revealed that hatching and developmental rates of nauplii and early copepodites were significantly impaired at such high CO_2

concentrations while older stages were not influenced by pCO_2 (Pedersen et al. 2013). Early *Calanus* life stages might thus be more sensitive to elevated pCO_2 than late copepodites and adults, as was also reported from *Acartia erythraea* (Kurihara et al. 2004). However, the nauplii of *C. finmarchicus* and *C. glacialis* hatch and develop in surface waters (reviewed e.g. by Falk-Petersen et al. 2009) and they will likely not encounter the high pCO_2 levels that have been shown to be detrimental for their development. *C. hyperboreus*, in contrast, spawn their eggs in deep waters during winter, and developing nauplii, which fuel on yolk and internal lipids in the beginning, then ascend to the surface (Conover 1988, Hirche and Niehoff 1996). During their early development, *C. hyperboreus* nauplii may thus be exposed to high levels of CO_2 via leakages from sub-seabed carbon storage sites, and as a consequence, less copepodites and adults might develop.

4.1.1.2 Diapausing copepods

The present study is the first one to evaluate the effects of elevated seawater pCO_2 on diapausing copepods. During their resting phase, climate change induced stress might be especially harmful to the copepods as they are not able to compensate for elevated energetic needs via increased food uptake (Saba et al. 2012). Our long-term experiment (17 weeks) on *C. hyperboreus* females did, however, not indicate that elevated pCO_2 induced stress to the resting copepods, as neither respiration nor body mass nor gonad development were affected (**Publication I**, Fig. 4.1). In addition, the hemolymph pH was measured to study if decreasing seawater pH will affect the extracellular acid-base status of the copepods. The average pH_e of diapausing *C. hyperboreus* females ranged between 4.7 and 6.6 and did not differ significantly between control (390 µatm) and high pCO_2 treatments (3000 µatm) (Fig. 4.3a). The pH in the hemolymph was thus even lower than the pH of the high CO_2 experimental water (pH 7.2). A similar phenomenon was found in Antarctic copepods. Sartoris et al. (2010) reported that diapausing *Calanoides acutus* and *Rhincalanus gigas* accumulated ammonia, which might aid to achieve neutral buoyancy at depth. As ammonia is toxic, the authors hypothesized that the copepods would down-regulate their extracellular pH in order to favor the formation of less toxic ammonium ions, and that such a drop in pH_e might also induce metabolic depression during diapause. Just recently, Schründer et al. (2013) confirmed that the mean hemolymph pH in *R. gigas* and *C. acutus* ranged between 5.7 and 7.3 during

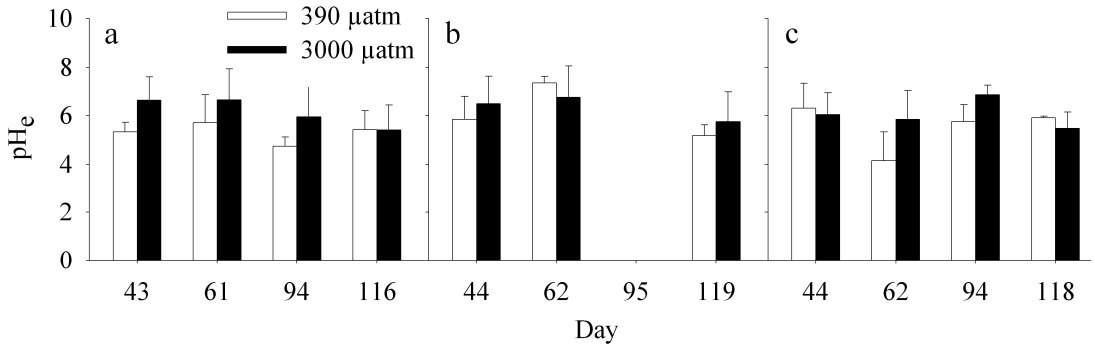

Fig. 4.3 Hemolymph pH (pH_e) of *Calanus hyperboreus* females incubated at (a) 0 °C, (b) 5 °C and (c) 10 °C at different CO_2 concentrations (390 µatm: white bars; 3000 µatm: black bars).

diapause, which is similar to the pH_e measured in *C. hyperboreus* (Fig. 4.3). In non-diapausing *Paraeuchaeta antarctica* and *Calanus propinquus*, in contrast, pH_e was 7.6 to 8.0 (Schründer et al. 2013). Due to their naturally low pH_e, diapausing *C. hyperboreus* and probably also other diapausing copepod species may thus not be challenged by the threats of elevated seawater pCO_2, even at very low pH conditions that might occur in the deep sea due to leakages from gas reservoirs (cf. Hall-Spencer et al. 2008, Cigliano et al. 2008).

In summary, predicted changes in seawater pCO_2 due to OA or leakages from sub-seabed storage sites will likely not affect late copepodites and adults of *C. hyperboreus*, *C. glacialis* and *C. finmarchicus*, neither during active nor during diapausing phases. The performance of young *C. hyperboreus* life stages might, however, be impaired when they develop in the surrounding of potential deep water CO_2 leakage sites.

4.1.2 Indirect effects

As key herbivores, the Arctic *Calanus* spp. may be influenced by indirect effects of OA via changes in the abundance or chemical composition of their prey algae. The main prey items of *C. finmarchicus*, *C. glacialis* and *C. hyperboreus* are diatoms, cryptophytes and haptophytes (e.g. Harris et al. 2000; Søreide et al. 2008). An elevated seawater pCO_2 can significantly reduce the nutritional quality of algae. In the diatom *Phaeodactylum tricornutum*, the C:N ratio increased at elevated CO_2 concentrations (Li et al. 2012). In *Nitzschia lecointei* (diatom), the total fatty acid content declined when exposed to OA (Torstensson et al. 2013), and in *Thalassiosira pseudonana* (diatom) both the total fatty acid content and the ratio of polyunsaturated to saturated fatty acids

decreased (Rossoll et al. 2012). This might have consequences for organisms feeding on the algae, resulting e.g. in lower fitness (Rossoll et al. 2012). In addition, results from a mesocosm study in an Arctic fjord at Svalbard indicate that OA might change the algal community composition: while the total amount of polyunsaturated fatty acids in the water column did not change significantly with pCO_2 in this experiment (Leu et al. 2013), diatoms decreased in abundance at high pCO_2, whereas picophytoplankton growth was favored under these conditions (Brussaard et al. 2013, Leu et al. 2013, Schulz et al. 2013). Such a shift to smaller food organisms at elevated pCO_2 might affect *Calanus* spp., as picoplankton is too small to be successfully grazed on by the copepods (Marshall and Orr 1955b), while competing organisms which are able to feed on picoplankton species, such as appendicularians, might benefit (Sommer et al. 2002).

Large-scale mesocosm studies tackling indirect effects of OA on zooplankton organisms are still rare, reflecting the high logistical effort and financial expense as compared to small-scale laboratory studies (Riebesell et al. 2010). However, mesocosm studies are powerful tools to investigate the interactions of three or more trophic levels under changing climatic conditions (Riebesell et al. 2010). In a mesocosm study at Svalbard, de Kluijver et al. (2013) found decreased rates of ^{13}C incorporation in *Calanus* spp. at elevated pCO_2, indicating that grazing rates decreased under these conditions. The authors speculated that this might be indirectly caused by OA via reduced production of mixotrophs or altered food quality at the high CO_2 levels (de Kluijver et al. 2013, Leu et al. 2013). Our laboratory studies have shown that the food uptake of *C. finmarchicus* and *C. glacialis* is not directly affected by OA (**Publication II**), and it is thus likely that indeed indirect effects were responsible for the reduced grazing rates found in the mesocosm experiment by de Kluijver et al. (2013).

During the SOPRAN CO_2 enrichment mesocosm study in 2011 in Bergen, Norway, grazing experiments with natural phytoplankton communities from the mesocosms were conducted in cooperation with J. R. Bermúdez Monsalve (GEOMAR) to test if food uptake of *C. finmarchicus* changes at elevated pCO_2 due to altered phytoplankton abundance. Unfortunately, the samples degraded during storage, and thus no data on indirect OA effects on grazing are available. The body mass of *C. finmarchicus* CV which we collected from two mesocosms (280 and 3000 µatm) for measuring grazing activity (**Publication II**) suggests that food uptake and assimilation might have been

indirectly affected during the first two weeks of the experiment, as the C content was significantly lower in copepods from the high as compared to the low pCO_2 mesocosm (**Publication II**). Here, lower food quality in the high pCO_2 mesocosm due to changes in the phytoplankton community (J. R. Bermúdez Monsalve (GEOMAR), pers. comm.) might have caused the lower body mass. Including CV and females sampled weekly from all mesocosms in the analyses, and thus covering the entire range of pCO_2, however, did not result in significant correlations between body mass and pCO_2 on any of the samplings, neither in CV (Fig. 4.4d-e) nor in females (Fig. 4.4d-e). This suggests that the food quality did not decrease continuously with pCO_2 but that the food quality only at high pCO_2 might have been suboptimal on the particular days. This effect, however, did not persist until the end of the experiment, and as respiration, stage development and, in females, gonad maturation and egg production were also not affected by elevated CO_2 concentrations (**Publication III**, Fig. 4.1, 4.2), no clear indications were found here that OA had indirectly altered grazing rates of *C. finmarchicus* in the high pCO_2 mesocosms during the 33 day experiment.

It has been shown by Urabe and Waki (2009) and Rossoll et al. (2013) that indirect OA effects on zooplankton species due to altered food quality, which can occur in two-species laboratory incubation studies (Urabe et al. 2003, Urabe and Waki 2009, Rossoll et al. 2012), might be mitigated when the animals are able to feed on a mixed phytoplankton community. In a diverse community, the response of algae to OA will be species-specific, and zooplankton species will have the opportunity to select adequate food organisms (Rossoll et al. 2013). Under natural conditions, copepods might therefore be able to cope with changing food conditions, as indicated by the results gained from a mesocosm study at Svalbard (Niehoff et al. 2013) and from our mesocosm study in Bergen (**Publication III**). However, if OA does not only affect the nutritional quality of single species, but alters the size regime of the whole phytoplankton community, as found during the mesocosm experiment at Svalbard (Brussaard et al. 2013, Leu et al. 2013, Schulz et al. 2013), *Calanus* spp. might still become affected in the long term.

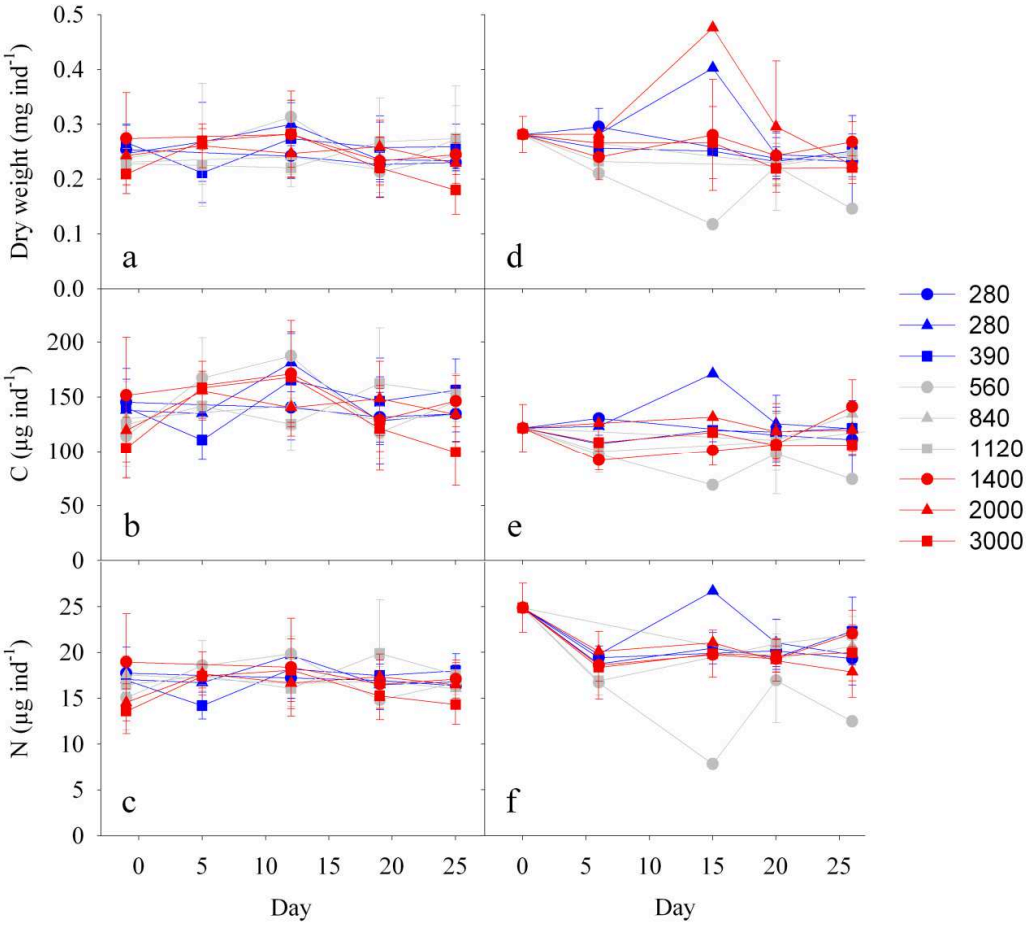

Fig. 4.4 *Calanus finmarchicus* CV dry weight (a), carbon content (b) and nitrogen content (c) and *C. finmarchicus* female dry weight (d), carbon content (e) and nitrogen content (f) during the SOPRAN mesocosm experiment. Numbers in the legend present initial CO_2. The number of measurements per data point ranged from 2 to 5 in *C. finmarchicus* CV and from 1 to 10 in *C. finmarchicus* females. For each measurement, 5 CV and 1 to 5 females were pooled.

4.1.3 Synergistic effects of elevated pCO_2 and temperature on Calanus

This study has shown that ocean acidification by itself will presumably not have major effects on the Arctic *Calanus* spp. However, OA does not occur in isolation, but in combination with other factors of anthropogenic climate change, one of which is ocean warming (Barnett et al. 2005, Solomon et al. 2007). To date, there have been only four studies published on synergistic effects of OA and ocean warming on copepods, which do not reveal consistent trends. Vehmaa et al. (2012) found that the positive effect of elevated temperatures on EPR and hatching success in *Acartia* sp. was mitigated by elevated seawater pCO_2. In contrast, the simultaneous increase of pH and temperature did not induce synergistic effects in egg production and hatching success of *A. clausi*

(Zervoudaki et al. 2014). Excretion was significantly stimulated in this species but it remains unclear how this will affect the copepods (Zervoudaki et al. 2014). During a small-scale mesocosm experiment, the increase in temperature of 3 °C did not induce CO_2 related changes in abundance in harpacticoid, cyclopoid and calanoid copepod assemblages (Troedsson et al. 2012). In *Calanus helgolandicus*, a close relative of the Arctic *Calanus* species which is distributed from the Mediterranean Sea to the coast of Scandinavia (Jaschnov 1970, Conover 1988, Bonnet et al. 2005), egg hatching was not affected by elevated pH and temperatures (Mayor et al. 2012).

During our study, the combined effects of elevated pCO_2 (3000 µatm) and water temperatures (5, 10 °C) on body mass, respiration, gonad development, extracellular acid-base status and mortality of diapausing *C. hyperboreus* females were determined. At 3000 µatm CO_2 and 5 °C, females had a significantly lower C and N content as compared to copepods from all other treatment groups after 17 weeks of exposure, which indicates that they suffered from elevated energetic demands which might affect the maximum diapause duration (**Publication I**). Such elevated energetic needs should have been accompanied by elevated metabolic rates, however, respiration was not affected, and also survival, gonad maturation and pH_e did not differ among copepods from different experimental conditions (**Publication I,** Fig. 4.3b, c). It is thus not possible to explain the detrimental effect on body mass at 5 °C and high pCO_2.

In general, temperature had a greater effect on diapausing *C. hyperboreus* than CO_2, altering respiration, gonad development and probably decreasing the total reproductive output (**Publication I**). Also other studies have shown that ocean warming might have a stronger impact on marine animals than ocean acidification (e.g. Byrne et al. 2009, Arnberg et al. 2012, Mayor et al. 2012, Troedsson et al. 2012). As our experiments have used temperatures that the copepods will most likely not encounter during their diapause phase in nature, however, it remains an open question whether more realistic temperature changes will still affect the performance of diapausing *Calanus*.

4.2 Marine copepod species in an acidified ocean

It is believed that the sensitivity of marine organisms to OA depends, among others, on the species' ability to regulate their extracellular and intracellular pH when the surrounding seawater pH changes. Uncompensated or incompletely compensated

decreases in body fluid pH might lead to metabolic depression (Reipschläger and Pörtner 1996, Pörtner et al. 1998), increased mortality (Miles et al. 2007) and affect processes such as feeding and growth (Appelhans et al. 2012) or calcification (Melzner et al. 2011). Species that are able to regulate their internal acid-base status generally seem to be much more tolerant to OA than non-regulators and calcifying species (Kroeker et al. 2010, Hale et al. 2011, Wittmann and Pörtner 2013). One of the most tolerant groups is the crustaceans (Kroeker et al. 2010, 2013, Wittmann and Pörtner 2013), which are strong regulators (Wheatly and Henry 1992, Widdicombe and Spicer 2008). A meta-analysis on the effects of OA on this taxonomic group revealed that the P_{50}, i.e. the CO_2 concentration at which 50 % of the species are affected, is 2086 µatm, which is considerably higher than in other tested groups, i.e. corals, echinoderms, molluscs and fishes (Wittmann and Pörtner 2013).

Copepods are an important crustacean group in marine pelagic ecosystems worldwide. To date, the effects of elevated pCO_2 on copepods have been investigated in 32 species, including 25 calanoid, 1 cyclopoid and 6 harpacticoid species (see Table A1 in the appendix). In addition, some studies on multi-species assemblages are available, in which copepod species were not differentiated (Table A1). Eight of the copepod species studied were from cold waters (< 5 °C), 16 from temperate regions (8 to 20 °C) and 7 species were kept above 20 °C. Exposure times to elevated pCO_2 were short (≤ 10 days) in most of the species studied (n = 19). Three species were exposed for 11 to 30 days, and in 10 species, the experiments lasted > 1 month. Sensitivities of copepods to OA were shown to be species specific (Table A1). Watanabe et al. (2006) have proposed from mortality experiments with extremely high CO_2 concentrations (up to 98,000 µatm) that deep-water and cold-water copepod species might be more tolerant to high pCO_2 than shallow-water and warm-water species. Cold and deep waters generally contain more CO_2 as compared to warm surface waters, and Lewis at al. (2013) suggested that copepods which experience relatively low pH values or high variations in pH during their life will be more robust to OA than copepods living predominantly in surface waters at relatively high pH and low pCO_2. The *Calanus* and *Neocalanus* spp. studied to date, which all undergo ontogenetic vertical migration (e.g. Hallberg and Hirche 1980, Miller et al. 1984, Hirche 1998, Li et al. 2004) and therefore inhabit cold deep waters during part of their lives, have indeed been shown to be generally robust to projected levels of OA (**Publication I-III**, Watanabe et al. 2006, Mayor et al. 2007,

2012, Zhang et al. 2011, Weydmann et al. 2012, Lewis et al. 2013). The potentially high capacities for acid-base regulation in diapausing species (Fig. 4.3; Schründer et al. 2013) might further contribute to their insensitivity to elevated pCO_2. In the warm water species *Tisbe battagliai*, on the other hand, a $pCO_2 \geq 394$ µatm ($pH \leq 7.82$) already affected growth and reproductive rates (Fitzer et al. 2012). However, most other species from warmer waters studied so far seem to be relatively insensitive to moderate changes in seawater pCO_2 (Tab. A1).

In order to evaluate the overall sensitivity of copepods to elevated seawater pCO_2, a statistical analysis including all published data was performed according to the method described in Wittmann and Pörtner (2013). In total, 6 out of 32 species studied (*Centropages tenuiremis*, *Acartia spinicauda*, *A. tonsa*, *Pseudocalanus elongatus* (all calanoids), *Tisbe battagliai* (harpacticoid)) were influenced at OA levels predicted for the year 2300 (**Publication III**, Dupont and Thorndyke 2008, Zhang et al. 2011, Fitzer et al. 2012, Li and Gao 2012; Tab. A1), and a statistically significant proportion of the copepods (≥ 18 %) is affected in the pCO_2 range 851-1370 µatm and beyond (Fisher's exact test, $p < 0.05$; Fig. 4.5). These results are in good agreement with data on the

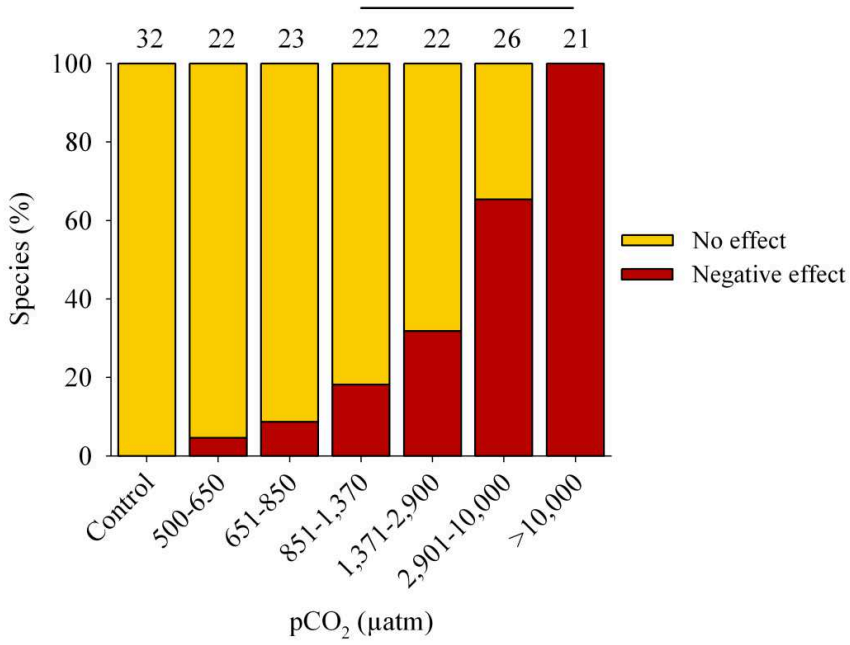

Fig. 4.5 Sensitivities of copepod species to ocean acidification. Data were processed after the method described in Wittmann and Pörtner (2013). Numbers above the columns denote the number of species analyzed within each CO_2 group. The bar above the columns denotes significant effects of pCO_2 as compared to the control group (Fisher's exact test, $p < 0.05$).

overall crustacean community that has been studied so far (Wittmann and Pörtner 2013), and the group of marine copepods will therefore likely be less sensitive to OA than calcifying species (corals, echinoderms, molluscs) and fishes, which were already affected in the pCO_2 range of 500-650 µatm (Wittmann and Pörtner 2013). OA might thus only marginally affect the copepod community as a whole and the *Calanus* community in particular, however, negative impacts of OA on few copepod species as well as effects on deep-sea or vertically migrating species due to possible CCS leakages could still lead to significant changes in the plankton community and the food web structure over longer time scales, as is also discussed for other plankton taxa (e.g. **Publication III**, Brussaard et al. 2013, Halsband and Kurihara 2013).

5 CONCLUSIONS AND FUTURE PERSPECTIVES

Calanus finmarchicus, *C. glacialis* and *C. hyperboreus* are major components of the lipid-based Arctic food web, and it is thus of high importance to understand how they will perform in the face of ongoing climate change. This study has shown that late copepodites and adults of the Arctic *Calanus* species are quite robust to the direct effects of elevated pCO_2 during both active and resting phases, even at CO_2 concentrations which exceed those projected to occur in surface waters during the next centuries. These results add to the picture that crustaceans in general are relatively tolerant to OA. However, this study has also shown that elevated pCO_2 in combination with elevated seawater temperatures might affect the *Calanus* species, and it is highly recommended that future studies should focus on synergistic effects of OA with other parameters of climate change. Furthermore, our CO_2/temperature study has emphasized the need for conducting long-term experiments with repeated measurements, including several aspects of the animal's ecology and physiology, to understand how climate change will influence the Arctic *Calanus* species and copepods in general.

The most threatening aspect of OA on the copepod community will likely be indirect effects. Changes in the phytoplankton community, be it in terms of nutritional quality or species abundance, might likely transfer to the copepods and affect e.g. their chemical body composition, growth and reproductive output. This will in turn not only have consequences for the copepod's population development, but it will also impact the role that *Calanus* species play in Arctic food webs, i.e. providing the link between primary production and higher trophic levels such as carnivorous zooplankton and fish. It is thus of high importance for future studies to further tackle the effects of OA (and also temperature) on whole plankton communities. Experiments with copepods feeding on low-quality food could indicate how the performance and nutritional quality of the copepods themselves will be affected in future decades. Despite logistically challenging, long-term mesocosm studies over several months would further complement results gained in multi-species laboratory studies to predict how OA-induced changes at the base of the food web will affect higher trophic levels.

6 REFERENCES

Albright, R. and Mason, B. (2013) Projected near-future levels of temperature and pCO_2 reduce coral fertilization success. *PLoS ONE* **8** (2), e56468. doi:10.1371/journal.pone.0056468.

Albritton, D. L., Meira Filho, L. G., Cubasch, U., Dai, X., Ding, Y., Griggs, D. J., Hewitson, B., Houghton, J. T., Isaksen, I., Karl, T., McFarland, M., Meleshko, V. P., Mitchell, J. F. B., Noguer, M., Nyenzi, B. S., Oppenheimer, M., Penner, J. E., Pollonais, S., Stocker, T. and Trenberth, K. E. (2001) Technical Summary. In: Houghton, J. T., Ding, Y., Griggs, D. J., Noguer, M., van der Linden, P. F., Dai, X., Maskell, K. and Johnson, C. A. (eds.) *Climate change 2001: The scientific basis.* Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge and New York.

Appelhans, Y. S., Thomsen, J., Pansch, C., Melzner, F. and Wahl, M. (2012) Sour times: seawater acidification effects on growth, feeding behaviour and acid-base status of *Asterias rubens* and *Carcinus maenas*. *Mar. Ecol. Prog. Ser.* **459**, 85-97.

Arnberg, M., Calosi, P., Spicer, J. I., Tandberg, A. H. S., Nilsen, M., Westerlund, S. and Bechmann, R. K. (2012) Elevated temperature elicits greater effects than decreased pH on the development, feeding and metabolism of northern shrimp (*Pandalus borealis*) larvae. *Mar. Biol.* **160**, 2037-2048.

Auel, H. and Hagen, W. (2002) Mesozooplankton community structure, abundance and biomass in the central Arctic Ocean. *Mar. Biol.* **140**, 1013-1021.

Auel, H., Klages, M. and Werner, I. (2003) Respiration and lipid content of the Arctic copepod *Calanus hyperboreus* overwintering 1 m above the seafloor at 2,300 m water depth in the Fram Strait. *Mar. Biol.* **143**, 275-282.

Barnett, T. P., Pierce, D. W., AchutaRao, K. M., Gleckler, P. J., Santer, B. D., Gregory, J. M. and Washington, W. M. (2005) Penetration of human-induced warming into the world's oceans. *Science* **309**, 284-287.

Beaugrand, G., Brander, K. M., Lindley, J. A., Souissi, S. and Reid, P. C. (2003) Plankton effect on cod recruitment in the North Sea. *Nature* **426**, 661-664.

Bellerby, R. G. J., Silyakova, A., Nondal, G., Slagstad, D., Czerny, J., de Lange, T. and Ludwig, A. (2012) Marine carbonate system evolution during the EPOCA Arctic pelagic ecosystem experiment in the context of simulated Arctic ocean acidification. *Biogeosciences Discuss.* **9**, 15541-15565.

Bibbi, R., Cleall-Harding, P., Rundle, S., Widdicombe, S. and Spicer, J. (2007) Ocean acidification disrupts induced defences in the intertidal gastropod *Littorina littorea*. *Biol. Lett.* **3**, 699-701.

Bonnet, D. and Carlotti, F. (2001) Development and egg production in *Centropages typicus* (Copepoda: Calanoida) fed different food types: a laboratory study. *Mar. Ecol. Prog. Ser.* **224**, 133-148.

Bonnet, D., Richardson, A., Harris, R., Hirst, A., Beaugrand, G., Edwards, M., Ceballos, S., Diekman, R., López-Urrutia, A., Valdes, L., Carlotti, F., Molinero, J. C., Weikert, H., Greve, W., Lucic, D., Albaina, A., Yahia, N. D., Umani, S. F., Miranda, A., dos Santos, A., Cook, K., Robinson, S. and Fernandez de Puelles, M. L. (2005) An overview of *Calanus helgolandicus* ecology in European waters. *Prog. Oceanogr.* **65**, 1-53.

Brussaard, C. P. D., Noordeloos, A. A. M., Witte, H., Collenteur, M. C. J., Schulz, K., Ludwig, A. and Riebesell, U. (2013) Arctic microbial community dynamics influenced by elevated CO_2 levels. *Biogeosciences* **10**, 719-731.

Byrne, M., Ho, M., Selvakumaraswamy, P., Nguyen, H. D., Dworjanyn, S. A. and Davis, A. R. (2009) Temperature, but not pH, compromises sea urchin fertilization and early development under near-future climate change scenarios. *Proc. R. Soc. B* **276**, 1883-1888.

Caldeira, K. and Wickett, M. E. (2003) Anthropogenic carbon and ocean pH. *Nature* **425**, 365.

Campbell, R. G., Runge, J. A. and Durbin, E. G. (2001) Evidence for food limitation of *Calanus finmarchicus* production rates on the southern flank of Georges Bank during April 1997. *Deep-Sea Res. II* **48**, 531-549.

Cigliano, M., Gambi, M. C., Rodolfo-Metalpa, R., Patti, F. P. and Hall-Spencer, J. M. (2010) Effects of ocean acidification on invertebrate settlement at volcanic CO_2 vents. *Mar. Biol.* **157**, 2489-2502.

Comeau, S., Alliouane, S. and Gattuso, J.-P. (2012) Effects of ocean acidification on overwintering juvenile Arctic pteropods *Limacina helicina. Mar. Ecol. Prog. Ser.* **456**, 279-284.

Conover, R. J. (1965) Notes on the molting cycle, development of sexual characters and sex ratio in *Calanus hyperboreus*. *Crustaceana* **8** (3), 308-320.

Conover, R. J. (1988) Comparative life histories in the genera *Calanus* and *Neocalanus* in high latitudes of the northern hemisphere. *Hydrobiologia* **167/168**, 127-142.

Conover, R. J. and Corner, E. D. S. (1968) Respiration and nitrogen excretion by some marine zooplankton in relation to their life cycles. *J. Mar. Biol. Ass. U.K.* **48**, 49-75.

Dawson, J. K. (1978) Vertical distribution of *Calanus hyperboreus* in the central Arctic Ocean. *Limnol. Oceanogr.* **23** (5), 950-957.

De Kluijver, A., Soetaert, K., Czerny, J., Schulz, K. G., Boxhammer, T., Riebesell, U. and Middelburg, J. J. (2013) A ^{13}C labeling study on carbon fluxes in Arctic plankton communities under elevated CO_2 levels. *Biogeosciences* **10**, 1425-1440.

Dlugokencky, E. and Tans, P. (2013) Trends in Atmospheric Carbon Dioxide: Recent Global CO_2. NOAA/ESRL, www.esrl.noaa.gov/gmd/ccgg/trends/, November 2013.

Doney, S. C., Fabry, V. J., Feely, R. A. and Kleypas, J. A. (2009) Ocean acidification: the other CO_2 problem. *Annu. Rev. Mar. Sci.* **1**, 169-192.

Dupont, S. and Thorndyke, M. C. (2008) Ocean acidification and its impact on the early life-history stages of marine animals. *CIESM Workshop Monographs* **36**, 89-97.

Dupont, S., Havenhand, J., Thorndyke, W., Peck, L. and Thorndyke, M. (2008) Near-future level of CO_2-driven ocean acidification radically affects larval survival and development in the brittlestar *Ophiothrix fragilis*. *Mar. Ecol. Prog. Ser*. **373**, 285-294.

Ellis, R. P., Bersey, J., Rundle, S. D., Hall-Spencer, J. M. and Spicer, J. I. (2009) Subtle but significant effects of CO_2 acidified seawater on embryos of the intertidal snail, *Littorina obtusata*. *Aquatic Biol*. **5**, 41-48.

Ericson, J. A., Ho, M. A., Miskelly, A., King, C. K., Virtue, P., Tilbrook, B. and Byrne, M. (2012) Combined effects of two ocean change stressors, warming and acidification, on fertilization and early development of the Antarctic echinoid *Sterechinus neumayeri*. *Polar Biol*. **35**, 1027-1034.

EU (2009) Directive 2009/31/EC of the European Parliament and of the Council of 23 April 2009 on the geological storage of carbon dioxide. *Off. J. Eur. Union* **L140**, 114-135.

Fabricius, K. E., Langdon, C., Uthicke, S., Humphrey, C., Noonan, S., De'ath, G., Okazaki, R., Muehllehner, N., Glas, M. S. And Lough, J. M. (2011) Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. *Nat. Clim. Change* **1**, 165-169.

Fabry, V. J., McClintock, J. B., Mathis, J. T. and Grebmeier, J. M. (2009) Ocean acidification at high latitudes: The bellwether. *Oceanography* **22** (4), 160-171.

Falk-Petersen, S., Pedersen, G., Kwasniewski, S., Hegseth, E. N. and Hop, H. (1999) Spatial distribution and life-cycle timing of zooplankton in the marginal sea ice zone of the Barents Sea during the summer melt season in 1995. *J. Plankton Res*. **21** (7), 1249-1264.

Falk-Petersen, S., Mayzaud, P., Kattner, G. And Sargent, J. R. (2009) Lipids and life strategy of Arctic *Calanus*. *Mar. Biol. Res.* **5**, 18-39.

Feely, R. A., Doney, S. C. and Cooley, S. R. (2009) Ocean acidification - present conditions and future changes in a high-CO_2 world. *Oceanography* **22** (4), 36-47.

Findlay, H. S., Kendall, M. A., Spicer, J. I. and Widdicombe, S. (2010) Post-larval development of two intertidal barnacles at elevated CO_2 and temperature. *Mar. Biol*. **157**, 725-735.

Fitzer, S. C., Caldwell, G. S., Close, A. J., Clare, A. S., Upstill-Goddard, R. C. and Bentley, M. G. (2012) Ocean acidification induces multi-generational decline in copepod naupliar production with possible conflict for reproductive resource allocation. *J. Exp. Mar. Biol. Ecol.* **418-419**, 30-36.

Fransz, H. G. and Gonzalez, S. R. (1997) Latitudinal metazoan plankton zones in the antarctic circumpolar current along 6°W during austral spring 1992. *Deep Sea Res. II*, **44** (1-2), 395-414.

Frost, B. W. (1972) Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. *Limnol. Oceanogr.* **17** (6), 805-815.

Gerlagh, R. and van der Zwaan, B. (2012) Evaluating uncertain CO_2 abatement over the very long term. *Environ. Model Assess*. **17**, 137-148.

Gislason, A. and Astthorsson, O. S. (1998) Seasonal variations in biomass, abundance and composition of zooplankton in the subarctic waters north of Iceland. *Polar Biol.* **20**, 85-94.

Gonzalez-Bernat, M. J., Lamare, M. and Barker, M. (2013) Effects of reduced seawater pH on fertilization, embryogenesis and larval development in the Antarctic seastar *Odontaster validus*. *Polar Biol*. **36** (2), 235-247.

Grainger, E. H. (1961) The copepods *Calanus glacialis* Jaschnov and *Calanus finmarchicus* (Gunnerus) in Canadian Arctic-Subarctic waters. *J. Fish. Res. BD. Can*. **18** (5), 663-678.

Gustavson, L. M. (2013) Effects of predicted ocean acidification scenarios on the early developmental stages of *Calanus finmarchicus*. A multigenerational study. Master Thesis, Norwegian University of Science and Technology. 90 pp.

Hale, R., Calosi, P., McNeill, L., Mieszkowska, N. and Widdicombe, S. (2011) Predicted levels of future ocean acidification and temperature rise could alter community structure and biodiversity in marine benthic communities. *Oikos* **120**, 661-674.

Hall-Spencer, J. M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., Fine, M., Turner, S. M., Rowley, S. J., Tedesco, D. and Buia, M.-C. (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature* **454**, 96-99.

Hallberg, E. and Hirche, H.-J. (1980) Differentiation of mid-gut in adults and over-wintering copepods of *Calanus finmarchicus* (Gunnerus) and *C. helgolandicus* Claus. *J. Exp. Mar. Biol. Ecol*. **48**, 283-295.

Halsband, C. and Kurihara, H. (2013) Potential acidification impacts on zooplankton in CCS leakage scenarios. *Mar. Pollut. Bull*. **73** (2), 495-503.

Harris, R. P., Irigoien, X., Head, R. N., Rey, C., Hygum, B. H., Hansen, B. W., Niehoff, B., Meyer-Harms, B. and Carlotti, F. (2000) Feeding, growth, and reproduction in the genus *Calanus*. *ICES J. Mar. Sci*. **57**, 1708-1726.

Havenhand, J. N., Buttler, F.-R., Thorndyke, M. C. and Williamson, J. E. (2008) Near-future levels of ocean acidification reduce fertilization success in a sea urchin. *Curr. Biol*. **18** (15), 651-652.

Hawkins, D. G. (2004) No exit: thinking about leakage from geologic carbon storage sites. *Energy* **29**, 1571-1578.

Head, E. J. H. and Conover, R. J. (1983) Induction of digestive enzymes in *Calanus hyperboreus*. *Mar. Biol. Lett*. **4**, 219-231.

Head, E. J. H. and Harris, L. R. (1985) Physiological and biochemical changes in *Calanus hyperboreus* from Jones Sound NWT during the transition from summer feeding to overwintering condition. *Polar Biol*. **4**, 99-106.

Hirche, H.-J. (1991) Distribution of dominant calanoid copepod species in the Greenland Sea during late fall. *Polar Biol*. **11**, 351-362.

Hirche, H.-J. (1997) Life cycle of the copepod *C. hyperboreus* in the Greenland Sea. *Mar. Biol.* **128**, 607-618.

Hirche, H.-J. (1998) Dormancy in three *Calanus* species (*C. finmarchicus*, *C. glacialis* and *C. hyperboreus*) from the North Atlantic. *Arch. Hydrobiol. Spec. Issues Advanc. Limnol*. **52**, 359-369.

Hirche, H.-J. and Mumm, N. (1992) Distribution of dominant copepods in the Nansen Basin, Arctic Ocean, in summer. *Deep-Sea Res*. **39** (Suppl. 2), 485-505.

Hirche, H.-J. and Kattner, G. (1993) Egg production and lipid content of *Calanus glacialis* in spring: indication of a food-dependent and food-independent reproductive mode. *Mar. Biol*. **117**, 615-622.

Hirche, H.-J. and Niehoff, B. (1996) Reproduction of the Arctic copepod *Calanus hyperboreus* in the Greenland Sea – field and laboratory observations. *Polar Biol*. **16**, 209-219.

Hirche, H.-J., Meyer, U. and Niehoff, B. (1997) Egg production of *C. finmarchicus*: effect of temperature, food and season. *Mar. Biol*. **127**, 609-620.

Holste, L. and Peck, M. A. (2006) The effects of temperature and salinity on egg production and hatching success of Baltic *Acartia tonsa* (Copepoda: Calanoida): a laboratory investigation. *Mar. Biol*. **148** (5), 1061-1070.

Håkedal, O. J. (2013) Effects on scope for growth due to elevated carbon dioxide in the copepod *C. finmarchicus*. Master Thesis, Norwegian University of Science and Technology. 65 pp.

Irigoien, X., Head, R. N., Harris, R. P., Cummings, D. and Harbour, D. (2000) Feeding selectivity and egg production of *Calanus helgolandicus* in the English Channel. *Limnol. Oceanogr*. **45** (1), 44-54.

Jaschnov, W. A. (1970) Distribution of *Calanus* species in the seas of the northern hemisphere. *Int. Revue ges. Hydrobiol*. **55** (2), 197-212.

Kawaguchi, S., Kurihara, H., King, R., Hale, L., Berli, T., Robinson, J. P., Ishida, A., Wakita, M., Virtue, P., Nicol, S. and Ishimatsu, A. (2011) Will krill fare well under southern ocean acidification? *Biol. Lett*. **7**, 288-291.

Kita, J., Kikkawa, T., Asai, T. and Ishimatsu, A. (2013) Effects of elevated pCO_2 on reproductive properties of the benthic copepod *Tigriopus japonicas* and gastropod *Babylonia japonica*. *Mar. Pollut. Bull*. **73**, 402-408.

Kraft, A., Berge, J., Varpe, Ø. And Falk-Petersen, S. (2013) Feeding in Arctic darkness: mid-winter diet of the pelagic amphipods *Themisto abyssorum* and *T. libellula*. *Mar. Biol.* **160**, 241-248.

Kroeker, K. J., Kordas, R. L., Crim, R. N. and Singh, G. G. (2010) Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecol. Lett*. **13**, 1419-1434.

Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M. And Gattuso, J.-P. (2013) Impacts of ocean acidification on marine organisms: quantifying sensitivities and interactions with warming. *Glob. Change Biol*. **19**, 1884-1896.

Kurihara, H. and Ishimatsu, A. (2008) Effects of high CO_2 seawater on the copepod (*Acartia tsuensis*) through all life stages and subsequent generations. *Mar. Pollut. Bull*. **56**, 1086-1090.

Kurihara, H., Shimode, S. and Shirayama, Y. (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.

Kurihara, H., Ishimatsu, A. and Shirayama, Y. (2007) Effects of elevated seawater CO_2 concentration on the meiofauna. *J. Mar. Sci. Technol*. *Taiwan* **15**, 17-22.

Kurihara, H., Matsui, M., Furukawa, H. and Hayashi, M. (2008) Long-term effects of predicted future seawater CO_2 conditions on the survival and growth of the marine shrimp *Palaemon pacificus*. *J. Exp. Mar. Biol. Ecol*. **367**, 41-46.

Lalli, C. M. and Parsons, T. R. (1997) Biological Oceanography: an introduction. 2nd edition. Elsevier, Oxford, UK and Burlington, MA, USA, 314 pp.

Le Quéré, C., Peters, G. P., Andres, R. J., Andrew, R. M., Boden, T., Ciais, P., Friedlingstein, P., Houghton, R. A., Marland, G., Moriarty, R., Sitch, S., Tans, P., Arneth, A., Arvanitis, A., Bakker, D. C. E., Bopp, L., Canadell, J. G., Chini, L. P., Doney, S. C., Harper, A., Harris, I., House, J. I., Jain, A. K., Jones, S. D., Kato, E., Keeling, R. F., Klein Goldewijk, K., Körtzinger, A., Koven, C., Lefèvre, N., Omar, A., Ono, T., Park, G.-H., Pfeil, B., Poulter, B., Raupach, M. R., Regnier, P., Rödenbeck, C., Saito, S., Schwinger, J., Segschneider, J., Stocker, B. D., Tilbrook, B., van Heuven, S., Viovy, N., Wanninkhof, R., Wiltshire, A., Zaehle, S. and Yue, C. (2013) Global carbon budget 2013. *Earth Syst. Sci. Data Discuss*. **6**, 689-760.

Leu, E., Daase, M., Schulz, K. G., Stuhr, A. and Riebesell, U. (2013) Effect of ocean acidification on the fatty acid composition of a natural plankton community. *Biogeosciences* **10**, 1143-1153.

Lewis, C. N., Brown, K. A., Edwards, L. A., Cooper, G. and Findlay, H. S. (2013) Sensitivity to ocean acidification parallels natural pCO_2 gradients experienced by Arctic copepods under winter sea ice. *PNAS* **110** (51), E4960-E4967.

Li, C., Sun, S., Wang, R. and Wang, X. (2004) Feeding and respiration rates of a planktonic copepod (*Calanus sinicus*) oversummering in Yellow Sea Cold Bottom Waters. *Mar. Biol*. **145**, 149-157.

Li, W. and Gao, K. (2012) A marine secondary producer respires and feeds more in a high CO_2 ocean. *Mar. Pollut. Bull*. **64**, 699-703.

Li, W., Gao, K. and Beardall, J. (2012) Interactive effects of ocean acidification and nitrogen-limitation on the diatom *Phaeodactylum tricornutum*. *PLoS ONE* **7** (12), e51590. doi:10.1371/journal.pone.0051590

Longhurst, A. R. (1985) The structure and evolution of plankton communities. *Prog. Oceanogr.* **15**, 1-35.

Marshall, S. M. and Orr, A. P. (1955a) The biology of a marine copepod: *C. finmarchicus* (Gunnerus). Oliver & Boyd, Edinburgh, London.

Marshall, S. M. and Orr, A. P. (1955b) On the biology of *C. finmarchicus*. VIII. Food uptake, assimilation and excretion in adult and stage V *Calanus*. *J. Mar. Biol. Ass. U.K*. **34**, 495-529.

Marshall, S. M., Nicholls, A. G. and Orr, A. P. (1935) On the biology of *C. finmarchicus*. Part VI. Oxygen consumption in relation to environmental conditions. *J. Mar. Biol. Ass. U.K.* **20**, 1-27.

Mayor, D. J., Matthews, C., Cook, K., Zuur, A. F. and Hay, S. (2007) CO_2-induced acidification affects hatching success in *Calanus finmarchicus*. *Mar. Ecol. Prog. Ser*. **350**, 91-97.

Mayor, D. J., Everett, N. R. and Cook, K. B. (2012) End of century ocean warming and acidification effects on reproductive success in a temperate marine copepod. *J. Plankton Res*. **34** (3), 258-262.

McConville, K., Halsband, C., Fileman, E. S., Somerfield, P. J. and Findlay, H. S. (2013) Effects of elevated CO_2 on the reproduction of two calanoid copepods. *Mar. Pollut. Bull.* **73** (2), 428-434.

McLaren, I. A. and Leonard, A. (1995) Assessing the equivalence of growth and egg production of copepods. *ICES J. Mar. Sci*. **52**, 397-408.

Melle, W. and Skjoldal, H. R. (1998) Reproduction and development of *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* in the Barents Sea. *Mar. Ecol. Prog. Ser*. **169**, 211-228.

Melzner, F., Stange, P., Trübenbach, K., Thomsen, J., Casties, I., Panknin, U., Gorb, S. N. and Gutowska, M. A. (2011) Food supply and seawater pCO_2 impact calcification and internal shell dissolution in the blue mussel *Mytilus edulis*. *PLoS ONE* **6**, e24223. doi:10.1371/journal.pone.0024223.

Metzger, R., Sartoris, F. J., Langenbuch, M. and Pörtner, H. O. (2007) Influence of elevated CO_2 concentrations on thermal tolerance of the edible crab *Cancer pagurus*. *J. Therm. Biol*. **32**, 144-151.

Michaelidis, B., Ouzounis, C., Paleras, A. and Pörtner, H. O. (2005) Effects of long-term moderate hypercapnia on acid-base balance and growth rate in marine mussels *Mytilus galloprovincialis*. *Mar. Ecol. Prog. Ser.* **293**, 109-118.

Michaud, J. and Taggart, C. T. (2011) Spatial variation in right whale food, *C. finmarchicus*, in the Bay of Fundy. *Endanger. Species Res*. **15**, 179-194.

Miles, H., Widdicombe, S., Spicer, J. I. and Hall-Spencer, J. (2007) Effects of anthropogenic seawater acidification on acid-base balance in the sea urchin *Psammechinus miliaris*. *Mar. Polut. Bull*. **54**, 89-96.

Miller, C. B., Frost, B. W., Batchelder, H. P., Clemons, M. J. and Conway, R. E. (1984) Life history of large, grazing copepods in a subarctic ocean gyre: *Neocalanus plumchrus*, *Neocalanus cristatus*, and *Eucalanus bungii* in the northeast Pacific. *Prog. Oceanogr*. **13**, 201-243.

Munday, P. L., Dixson, D. L., Donelson, J. M., Jones, G. P., Pratchett, M. S., Devitsina, G. V. and Døving, K. B. (2009) Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *PNAS* **106** (6), 1848-1852.

Munday, P. L., Dixson, D. L., McCormick, M. I., Meekan, M., Ferrari, M. C. O. and Chivers, D. P. (2010) Replenishment of fish populations is threatened by ocean acidification. *PNAS* **107** (29), 12930-12934.

Niehoff, B. (2003) Gonad morphology and oocyte development in *Pseudocalanus* spp. in relation to spawning activity. *Mar. Biol*. **143**, 759-768.

Niehoff, B. (2004) The effect of food limitation on gonad development and egg production of the planktonic copepod *Calanus finmarchicus*. J. Exp. Mar. Biol. Ecol. 307, 237-259.

Niehoff, B. (1998) The gonad morphology and maturation in Arctic *Calanus* species. *J. Mar. Syst*. **15**, 53-59.

Niehoff, B., Klenke, U., Hirche, H.-J., Irigoien, X., Head, R. and Harris, R. (1999) A high frequency time series at Weathership M, Norwegian Sea, during the 1997 spring bloom: the reproductive biology of *C. finmarchicus. Mar. Ecol. Prog. Ser*. **176**, 81-92.

Niehoff, B., Madsen, S. D., Hansen, B. W. and Nielsen, T. G. (2002) Reproductive cycles of three dominant *Calanus* species in Disko Bay, West Greenland. *Mar. Biol*. **140**, 567-576.

Niehoff, B., Schmithüsen, T., Knüppel, N., Daase, M., Czerny, J. and Boxhammer, T. (2013) Mesozooplankton community development at elevated CO_2 concentrations: results from a mesocosm experiment in an Arctic fjord. *Biogeosciences* **10**, 1391-1406.

O'Donnell, M. J., Hammond, L. M. and Hofmann, G. E. (2009) Predicted impact of ocean acidification on a marine invertebrate: elevated CO_2 alters response to thermal stress in sea urchin larvae. *Mar. Biol*. **156**, 439-446.

Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., Key, R. M., Lindsay, K., Maier-Reimer, E., Matear, R., Monfray, P., Mouchet, A., Najjar, R. G., Plattner, G.-K., Rodgers, K. B., Sabine, C. L., Sarmiento, J. L., Schlitzer, R., Slater, R. D., Totterdell, I. J., Weirig, M.-F., Yamanaka, Y. and Yool, A. (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **437**, 681-686.

Pascal, P.-Y., Fleeger, J. W., Galvez, F. and Carman, K. R. (2010) The toxicological interaction between ocean acidity and metals in coastal meiobenthic copepods. *Mar. Pollut. Bull*. **60**, 2201-2208.

Pedersen, S. A., Hansen, B. H., Altin, D. and Olsen, A. J. (2013) Medium-term exposure of the North Atlantic copepod *Calanus finmarchicus* (Gunnerus, 1770) to CO_2-acidified seawater: effects on survival and development. *Biogeosciences* **10**, 7481-7491.

Plourde, S. and Runge, J. A. (1993) Reproduction of the planktonic copepod *Calanus finmarchicus* in the Lower St. Lawrence Estuary: relation to the cycle of phytoplankton production and evidence for a *Calanus* pump. *Mar. Ecol. Prog. Ser*. **102**, 217-227.

Pörtner, H. O., Reipschläger, A. and Heisler, N. (1998) Acid-base regulation, metabolism and energetics in *Sipunculus nudus* as a function of ambient carbon dioxide level. *J. Exp. Biol.* **201**, 43-55.

Prokopchuk, I. and Sentyabov, E. (2006) Diets of herring, mackerel, and blue whiting in the Norwegian Sea in relation to *Calanus finmarchicus* distribution and temperature conditions. *ICES J. Mar. Sci*. **63**, 117-127.

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Raven, J., Caldeira, K., Elderfield, H., Hoegh-Guldberg, O., Liss, P., Riebesell, U., Shepherd, J., Turley, C. and Watson, A. (2005) Ocean acidification due to increasing atmospheric carbon dioxide. Policy document 12/05, The Royal Society, UK.

Reipschläger, A. and Pörtner, H. O. (1996) Metabolic depression during environmental stress: The role of extracellular *versus* intracellular pH in *Sipunculus nudus*. *J. Exp. Biol*. **199**, 1801-1807.

Reynaud, S., Leclercq, N., Romaine-Lioud, S., Ferrier-Pagès, C., Jaubert, J. and Gattuso, J.-P. (2003) Interacting effects of CO_2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral. *Glob. Change Biol.* **9**, 1660-1668.

Riebesell, U., Zondervan, I., Rost, B., Tortell, P. D., Zeebe, R. E. and Morel, F. M. M. (2000) Reduced calcification of marine plankton in response to increased atmospheric CO_2. *Nature* **407**, 364-367.

Riebesell, U., Lee, K. and Neijstgaard, J. C. (2010) Pelagic mesocosms. In: Riebesell, U., Fabry, V. J., Hansson, L. and Gattuso, J.-P. (eds.) *Guide to best practices for ocean acidification research and data reporting.* Publications Office of the European Union, Luxembourg, pp. 95-112.

Riebesell, U., Czerny, J., von Bröckel, K., Boxhammer, T., Büdenbender, J., Deckelnick, M., Fischer, M., Hoffmann, D., Krug, S. A., Lentz, U., Ludwig, A., Muche, R. and Schulz, K. G. (2013) Technical note: a mobile sea-going mesocosm system - new opportunities for ocean change research. *Biogeosciences* **10**, 1835-1847.

Rosa, R. and Seibel, B. A. (2008) Synergistic effects of climate-related variables suggest future physiological impairment in a top oceanic predator. *PNAS* **105** (52), 20776-20780.

Rossoll, D., Bermúdez, R., Hauss, H., Schulz, K. G., Riebesell, U., Sommer, U. and Winder, M. (2012) Ocean acidification-induced food quality deterioration constrains trophic transfer. *PLoS ONE* **7** (4), e34737. doi:10.1371/journal.pone.0034737.

Rossoll, D., Sommer, U. and Winder, M. (2013) Community interactions dampen acidification effects in a coastal plankton system. *Mar. Ecol. Prog. Ser.* **486**, 37-46.

Rubin, E., Meyer, L., de Coninck, H., Abanades, J. C., Akai, M., Benson, S., Caldeira, K., Cook, P., Davidson, O., Doctor, R., Dooley, J., Freund, P., Gale, J., Heidug, W., Herzog, H., Keith, D., Mazzotti, M., Metz, B., Osman-Elasha, B., Palmer, A., Pipatti, R., Smekens, K., Soltanieh, M., Thambimuthu, K. and van der Zwaan, B. (2005) Technical summary. In: Metz, B., Davidson, O., de Coninck, H., Loos, M. and Meyer, L. (eds.) *IPCC Special Report on Carbon Dioxide Capture and Storage*. Cambridge University Press, Cambridge, UK and New York, NY, USA.

Runge, J. A. (1988) Should we expect a relationship between primary production and fisheries? The role of copepod dynamics as a filter of trophic variability. *Hydrobiologia* **167/168**, 61-71.

Runge, J. A. and Roff, J. C. (2000) The measurement of growth and reproductive rates. In: Harris, R. P., Wiebe, P. H., Lenz, J., Skjoldal, H. R. and Huntley, M. (eds.) *ICES Zooplankton Methodology Manual*. Academic Press, San Diego, pp. 401-454.

Saba, G. K., Schofield, O., Torres, J. J., Ombres, E. H. and Steinberg, D. K. (2012) Increased feeding and nutrient excretion of adult Antarctic krill, *Euphausia superba*, exposed to enhanced carbon dioxide (CO_2). *PLoS ONE* **7** (12), e52224. doi:10.1371/journal.pone.0052224.

Sartoris, F. J., Thomas, D. N., Cornils, A. and Schnack-Schiel, S. B. (2010) Buoyancy and diapause in Antarctic copepods: The role of ammonium accumulation. *Limnol. Oceanogr.* **55** (5), 1860-1864.

Saumweber, W. J. and Durbin, E. G. (2006) Estimating potential diapause duration in *Calanus finmarchicus*. *Deep-Sea Res. II* **53**, 2597-2617.

Schrag, D. P. (2009) Storage of carbon dioxide in offshore sediments. *Science* **325**, 1658-1659.

Schründer, S., Schnack-Schiel, S. B., Auel, H. and Sartoris, F. J. (2013) Control of diapause by acidic pH and ammonium accumulation in the hemolymph of Antarctic copepods. *PLoS ONE* **8** (10), e77498. doi:10.1371/journal.pone.0077498.

Schulz, K. G., Bellerby, R. G. J., Brussaard, C. P. D., Büdenbender, J., Czerny, J., Engel, A., Fischer, M., Koch-Klavsen, S., Krug, S. A., Lischka, S., Ludwig, A., Meyerhöfer, M., Nondal, G., Silyakova, A., Stuhr, A. and Riebesell, U. (2013) Temporal biomass dynamics of an Arctic plankton bloom in response to increasing levels of atmospheric carbon dioxide. *Biogeosciences* **10**, 161–180.

Shepherd, J. G. (2009) Geoengineering the climate: science, governance and uncertainty. *RS Policy document* **10/09**, The Royal Society, London.

Solomon, S., Quin, D., Manning, M., Alley, R. B., Berntsen, T., Bindoff, N. L., Chen, Z., Chidthaisong, A., Gregory, J. M., Hegerl, G. C., Heimann, M., Hewitson, B., Hoskins, B. J., Joos, F., Jouzel, J., Kattsov, V., Lohmann, U., Matsuno, T., Molina, M., Nicholls, N., Overpeck, J., Raga, G., Ramaswamy, V., Ren, J., Rusticucci, M., Somerville, R., Stocker, T. F., Whetton, P., Wood, R. A. and Wratt, D. (2007) Technical summary. In: Solomon, S., Quin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M. and Miller, H. L. (eds.) *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Soltwedel, T., Bauerfeind, E., Bergmann, M., Budaeva, N., Hoste, E., Jaeckisch, N., von Juterzenka, K., Matthiessen, J., Mokievsky, V., Nöthig, E.-M., Quéric, N. V., Sablotny, B., Sauter, E., Schewe, I., Urban-Malinga, B., Wegner, J., Wlodarska-Kowalczuk, M. and Klages, M. (2005) HAUSGARTEN: Multidisciplinary investigations at a deep-sea, long-term observatory in the Arctic Ocean. *Oceanography* **18** (3), 46-61.

Somavilla, R., Schauer, U. and Budéus, G. (2013) Increasing amount of Arctic Ocean deep waters in the Greenland Sea. *Geophys. Res. Lett.* **40**, 4361-4366.

Sommer, U., Stibor, H., Katechakis, A., Sommer, F. and Hansen, T. (2002) Pelagic food web configurations at different levels of nutrient richness and their implications for the ratio fish production:primary production. *Hydrobiologia* **484**, 11-20.

Stocker, T. F., Qin, D., Plattner, G.-K., Alexander, L. V., Allen, S. K., Bindoff, N. L., Bréon, F.-M., Church, J. A., Cubasch, U., Emori, S., Forster, P., Friedlingstein, P., Gillett, N., Gregory, J. M., Hartmann, D. L., Jansen, E., Kirtman, B., Knutti, R., Krishna Kumar, K., Lemke, P., Marotzke, J., Masson-Delmotte, V., Meehl, G. A., Mokhov, I. I., Piao, S., Ramaswamy, V., Randall, D., Rhein, M., Rojas, M., Sabine, C., Shindell, D., Talley, L. D., Vaughan, D. G. and Xie, S.-P. (2013) Technical Summary. In: Stocker, T. F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V. and Midgley, P. M. (eds.) *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, **in press**.

Stumpp, M., Hu, M., Casties, I., Saborowski, R., Bleich, M., Melzner, F. and Dupont, S. (2013) Digestion in sea urchin larvae impaired under ocean acidification. *Nat. Clim. Change* **3** (12), 1044-1049.

Swalethorp, R., Kjellerup, S., Dünweber, M., Nielsen, T. G., Møller, E. F., Rysgaard, S. and Hansen, B. W. (2011) Grazing, egg production, and biochemical evidence of differences in the life strategies of *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* in Disko Bay, western Greenland. *Mar. Ecol. Prog. Ser.* **429**, 125-144.

Søreide, J. E., Falk-Petersen, S., Hegseth, E. N., Hop, H., Carroll, M. L., Hobson, K. A. and Blachowiak-Samolyk, K. (2008) Seasonal feeding strategies of *Calanus* in the high-Arctic Svalbard region. *Deep-Sea Res. II* **55**, 2225-2244.

Tande, K. S., Hassel, A. and Slagstad, D. (1985) Gonad maturation and possible life cycle strategies in *Calanus finmarchicus* and *Calanus glacialis* in the northwestern part of the Barents Sea. In: Gray, J. S. and Christiansen, M. E. (eds.) *Biology of polar regions and effect of stress on marine organisms.* J. Wiley & Sons Ltd., New York, pp. 141-155.

Thistle, D., Carman, K. R., Sedlacek, L., Brewer, P. G., Fleeger, J. W. and Barry, J. P. (2005) Deep-ocean, sediment-dwelling animals are sensitive to sequestered carbon dioxide. *Mar. Ecol. Prog. Ser.* **289**, 1-4.

Thistle, D., Sedlacek, L., Carman, K. R., Fleeger, J. W., Brewer, P. G. and Barry, J. P. (2006) Simulated sequestration of industrial carbon dioxide at a deep-sea site: Effects on species of harpacticoid copepods. *J. Exp. Mar. Biol. Ecol.* **330**, 151-158.

Thistle, D., Sedlacek, L., Carman, K. R., Fleeger, J. W., Brewer, P. G. and Barry, J. P. (2007) Exposure to carbon dioxide-rich seawater is stressful for some deep-sea species: an *in situ*, behavioral study. *Mar. Ecol. Prog. Ser.* **340**, 9-16.

Troedsson, C., Bouquet, J.-M., Lobon, C. M., Novac, A., Nejstgaard, J. C., Dupont, S., Bosak, S., Jakobsen, H. H., Romanova, N., Pankoke, L. M., Isla, A., Dutz, J., Sazhin, A. F. and Thompson, E. M. (2012) Effects of ocean acidification, temperature and nutrient regimes on the appendicularian *Oikopleura dioica*: a mesocosm study. *Mar. Biol*. **160**, 2175-2187.

Torstensson, A., Hedblom, M., Andersson, J., Andersson, M. X. and Wulff, A. (2013) Synergism between elevated pCO_2 and temperature on the Antarctic sea ice diatom *Nitzschia lecointei*. *Biogeosciences* **10**, 6391-6401.

Urabe, J., Togari, J. and Elser, J. J. (2003) Stoichiometric impacts of increased carbon dioxide on a planktonic herbivore. *Glob. Change Biol*. **9**, 818-825.

Urabe, J. and Waki, N. (2009) Mitigation of adverse effects of rising CO_2 on a planktonic herbivore by mixed algal diets. *Glob. Change Biol*. **15**, 523-531.

Veehma, A., Brutemark, A. and Engström-Öst, J. (2012) Maternal effects may act as an adaptation mechanism for copepods facing pH and temperature changes. *PLoS ONE* **7** (10), e48538. doi:10.1371/journal.pone.0048538.

Walther, K., Sartoris, F. J., Bock, C. and Pörtner, H. O. (2009) Impact of anthropogenic ocean acidification on thermal tolerance of the spider crab *Hyas araneus*. *Biogeosciences* **6**, 2207-2215.

Watanabe, Y., Yamaguchi, A., Ishida, H., Harimoto, T., Suzuki, S., Sekido, Y., Ikeda, T., Shirayama, Y., Takahashi, M. M., Ohsumi, T. and Ishizaka, J. (2006) Lethality of increasing CO_2 levels on deep-sea copepods in the western North Pacific. *J. Oceanogr*. **62**, 185-196.

Węsławski, J. M., Stempniewicz, L., Mehlum, F. and Kwaśniewski, S. (1999) Summer feeding strategy of the little auk (*Alle alle*) from Bjørnøya, Barents Sea. *Polar Biol*. **21**, 129-134.

Wishner, K., Durbin, E., Durbin, A., Macaulay, M., Winn, H. and Kenney, R. (1988) Copepod patches and right whales in the Great South Channel off New England. *Bull. Mar. Sci*. **43** (3), 825-844.

Weydmann, A., Søreide, J. E., Kwasniewski, S. and Widdicombe, S. (2012) Influence of CO_2-induced acidification on the reproduction of a key Arctic copepod *Calanus glacialis*. *J. Exp. Mar. Biol. Ecol*. **428**, 39-42.

Wheatly, M. G. and Henry, R. P. (1992) Extracellular and intracellular acid-base regulation in crustaceans. J. Exp. Zool. 263 (2), 127-142.

Widdicombe, S. and Needham, H. R. (2007) Impact of CO_2-induced seawater acidification on the burrowing activity of *Nereis virens* and sediment nutrient flux. *Mar. Ecol. Prog. Ser*. **341**, 111-122.

Widdicombe, S. and Spicer, J. I. (2008) Predicting the impact of ocean acidification on benthic biodiversity: What can animal physiology tell us? *J. Exp. Mar. Biol. Ecol*. **366**, 187-197.

Wittmann, A. C. and Pörtner, H.-O. (2013) Sensitivities of extant animal taxa to ocean acidification. *Nat. Clim. Change* **3** (11), 995-1001.

Wold, A., Jæger, I., Hop, H., Gabrielsen, G. W. and Falk-Petersen, S. (2011) Arctic seabird food chains explored by fatty acid composition and stable isotopes in Kongsfjorden, Svalbard. *Polar Biol*. **34**, 1147-1155.

Yamada, Y. and Ikeda, T. (1999) Acute toxicity of lowered pH to some oceanic zooplankton. *Plankton Biol. Ecol.* **46** (1), 62-67.

Zervoudaki, S., Frangoulis, C., Giannoudi, L. and Krasakopoulou, E. (2014) Effects of low pH and raised temperature on egg production, hatching and metabolic rates of a Mediterranean copepod species (*Acartia clausi*) under oligotrophic conditions. *Mediterr. Mar. Sci.* **15** (1), 74-83.

Zhang, D., Li, S., Wang, G. and Guo, D. (2011) Impacts of CO_2-driven seawater acidification on survival, egg production rate and hatching success of four marine copepods. *Acta Oceanol. Sin*. **30** (6), 86-94.

Zhang, D., Li, S., Wang, G., Guo, D., Xing, K. and Zhang, S. (2012) Biochemical responses of the copepod *Centropages tenuiremis* to CO_2-driven acidified seawater. *Water Sci. Technol*. **65** (1), 30-37.

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Table A1 Effects of elevated pCO_2 on copepods. CI-CVI = copepodite stages I-VI; NI-NVI = nauplii stages I-VI; ♀ = adult females; ♂ = adult males; d = days; h = hours; LCS = full life-cycle study; MGS = multi-generational study; mo = months; MS = mesocosm study, Temp = incubation temperature; ↓ = decrease; ↔ = no effect; ↑ = increase; p = parabolic response. Depicted increases or decreases in response do not necessarily occur at all tested pCO_2 levels, but at least at one of them.

Copepod species/life stage	pCO_2 (µatm)	pH	Exposure time	Temp. (°C)	Response		Reference	Remarks
Calanoida								
Family Acartiidae Sars, 1903								
Acartia clausi adults/eggs	824	7.83	5 d	16	↔	egg production	Zervoudaki et al. (2014)	
					↔	hatching success		
					↔	adult respiration		
					↓	adult excretion		
Acartia erythraea ♀/eggs/nauplii	2380/5380/10,380	7.31/7.00/6.82	8 d	27	↔	female survival	Kurihara et al. (2004)	
					↓	egg production		
					↓	hatching success		
					↓	nauplii mortality		
Acartia pacifica ♀	800/2000/ 5000/10,000	7.84/7.39/ 7.19/6.92	8 d	18	↔	survival	Zhang et al. (2011)	
					↔	egg production		
Acartia spinicauda ♀/eggs	800/2000/ 5000/10,000	7.84/7.39/ 7.19/6.92	8 d	24	↓	survival	Zhang et al. (2011)	
					↓	egg production		
	2000/10,000	7.39/6.92			↓	hatching success		
Acartia steueri ♀	2380/10,380	7.4/6.84	8 d	24	↔	survival	Kurihara et al. (2004)	
					↓	egg production		
Acartia tonsa NI-CVI		7.9/7.7			↔	survival	Dupont and Thorn-dyke (2008)	MGS
					↑	generation time[1]		
Acartia tonsa NI-CVI, eggs	760	~ 7.9	10 d	18	↑	developmental time	Rossoll et al. (2012)	LCS; indirect effects via altered food
					↓	% PUFA/MUFA (♀)		
					↑	% SFA (♀)		
					↓	egg production		
					↔	nauplii morphology		

[1]only in generation one; [2] initial pCO_2/pH at the start of the study; PUFA: polyunsaturated fatty acids; MUFA: monounsaturated fatty acids; SFA: saturated fatty acids.

Table A1 (continued).

Copepod species	pCO_2 (µatm)	pH	Exposure time	Temp. (°C)	Response		Reference	Remarks
Acartia tonsa NII-CVI, eggs	840/1120/ 1400/4000[1]		29 d	18	↔	stage development	Rossoll et al. (2013)	MS
					↔	egg production		
					↔	hatching success		
Acartia tsuensis NI-CVI, eggs	2380	7.32	~ 30 d	25	↔	female survival	Kurihara and Ishi- matsu (2008)	MGS
					↔	development		
					↔	egg production		
					↓	hatching success[2]		
Acartia sp. (A. bifilosa) ♀*/eggs*		7.4	5 d	17	↔	egg production	Veehma et al. (2012)	
					↔	hatching success		
Acartia spp. NI-CVI	280 - 3000[1]	8.2 - 7.22	33 d	6.9-10	↔	abundance	this study	MS
Family Aetideidae Giesbrecht, 1892								
Gaidius variabilis	1100/8800		≤ 10 d	3	↓	survival	Watanabe et al. (2006)	
Family Calanidae Dana, 1849								
Calanus finmarchicus CV	1120/3000	7.67/7.30	13 d	5	↔	feeding	this study	
					↔	body mass		
Calanus finmarchicus CV	3000[1]	7.2[1]	33 d	6.9-10	↔	feeding	this study	MS
	1400/3000[1]	7.5/7.2[1]			↔	respiration		
	280 - 3000[1]	8.2 - 7.2[1]			↔	body mass		
Calanus finmarchicus ♀	280 - 3000[1]	8.2 - 7.2[1]	33 d	6.9-10	↔	body mass	this study	MS
Calanus finmarchicus CI-CVI	280 - 3000[1]	8.2 - 7.2[1]	33 d	6.9-10	↔	abundance	this study	MS
					↔	stage development		
Calanus finmarchicus ♀/eggs	8000	6.95	5 d	8.8	↔	body mass	Mayor et al. (2007)	
					↔	egg production		
					↓	hatching success		
Calanus finmarchicus CV/♀/♂		6.7	2 d	12	↔	survival	Marshall et al. (1935)	
		7.3	4 h	12	↔	respiration		

[1]initial pCO_2/pH at the start of the study; [2]only when all three generations are included, no effect for each separate generation

Table A1 (continued).

Copepod species	pCO_2 (µatm)	pH	Exposure time	Temp (°C)	Response		Reference	Remarks
Calanus finmarchicus eggs, NI-CVI	3300/7300/9700	7.31/6.97/6.85	28 d	10	↓	survival	Pedersen et al. (2013)	LCS
					↑	developmental time		
					↔	prosome length		
					↔	fat content		
Calanus finmarchicus eggs, NI-CVI	1080/2080/3080	7.64/7.33/7.15	136 d	10	↑	developmental time[2]	Gustavson (2013)	MGS
					↔	population density		
Calanus finmarchicus eggs, NI-CVI	1080/2080/3080	7.6/7.3/7.2	~ 5 mo	10	↑	developmental time[3]	Håkedal (2013)	MGS
Calanus finmarchicus CV	1080/2080/3080	7.6/7.3/7.2	~ 5 mo	10	↔	body mass	Håkedal (2013)	MGS
					↔	respiration		
					*	grazing rate		
					p	scope for growth		
Calanus finmarchicus/Pseudo-calanus elongatus NI-NVI	280 - 3000[1]	8.2 - 7.2[1]	33 d	6.9-10	↓	abundance	this study	MS
					↔	stage development		
Calanus glacialis CV	3000	7.24	62 d	0	↑	survival	this study	
					↔	respiration		
					↔	body mass		
Calanus glacialis CV	1120/3000	7.55/7.24	16 d	0	↔	feeding	this study	
					↔	body mass		
Calanus glacialis ♀/eggs		7.6/6.9	9 d	-1	↔	female mortality	Weydmann et al. (2012)	
					↔	egg production		
					↓	hatching success		
					↑	time until egg hatching		
Calanus glacialis/hyperboreus adults	700/1000	7.8/7.6	7 d	-1.7	↔	survival	Lewis et al. (2013)	
Calanus helgolandicus eggs	1000	7.77	72 h	8	↔	hatching success	Mayor et al. (2012)	

[1]initial pCO_2/pH at the start of the study; only NV, NVI and CI; [2] only NV, NVI and CI; [3]only NVI and CIV; *significant effect, but no consistent trend

Table A1 (continued).

Copepod species	pCO_2 (µatm)	pH	Exposure time	Temp (°C)	Response		Reference	Remarks
Calanus hyperboreus CV/♀	3000	7.23	63 - 119 d	0	↔	survival	this study	diapause
					↔	respiration		
					↔	body mass		
					↔	gonad development (♀)		
					↔	hemolymph pH (♀)		
Calanus pacificus	3500 - 22,000		4 d	3	↓	survival	Watanabe et al. (2006)	
Calanus sinicus	800/2000/ 5000/10,000	7.84/7.39/ 7.19/6.92	8 d	16	↔	female survival	Zhang et al. (2011)	
					↓	egg production		
Calanus spp.	185 - 1420[1]	~8.3 - 7.5[1,2]	30 d	2-6[3]	↓	grazing rate	de Kluijver et al. (2013)	MS
Calanus spp. CI-CVI	185 - 1420[1]	~8.3 - 7.5[1,2]	30 d	2-6[3]	↔	abundance	Niehoff et al. (2013)	MS
					↔	stage development		
nauplii >250 µm (*Calanus* spp.?)	700/1000	7.8/7.6	7 d	-1.7	↓	survival	Lewis et al. (2013)	
Neocalanus cristatus	3500 - 22,000		10 d	3	↓	survival	Watanabe et al. (2006)	
Family Centropagidae Giesbrecht, 1893								
Centropages tenuiremis	1000	7.83	24 h	20	↑	respiration	Li and Gao (2012)	
					↓	feeding		
			90 h	20	↑	respiration		
					↔	feeding		
Centropages tenuiremis ♀/eggs	800/2000/ 5000/10,000	7.84/7.39/ 7.19/6.92	8 d	21	↓	female survival	Zhang et al. (2011)	
					↓	egg production		
	2000/10,000	7.39/6.92	8 d	21	↓	hatching success		

[1]initial pCO_2/pH at the start of the study; [2]data after Bellerby et al. (2012); [3]data after Schulz et al. (2013)

Table A1 (continued).

Copepod species	pCO_2 (µatm)	pH	Exposure time	Temp (°C)	Response		Reference	Remarks
Centropages tenuiremis ♀	800/2000/ 5000/10,000	7.82/7.37/ 7.16/6.92	4 d	21	↔	AchE activity	Zhang et al. (2012)	
					↔	ATPase activity		
					↑	GPx activity		
					↔	GST activity		
					*	SOD activity		
					*	GSH activity		
					↔	GSH/GSSR ratio		
Centropages typicus ♀/eggs	480/620/750	7.97/7.85/7.78	4 d	14.7	↔	egg production	McConville et al. (2013)	
					↔	hatching success		
	9830	6.71	4 d	14.7	↓	egg production		
					↓	hatching success		
Centropages spp. CI-CVI	280 - 30001	8.2 - 7.2[1]	33 d	6.9-10	↔	abundance	this study	MS
Family Clausocalanidae Giesbrecht, 1893								
Pseudocalanus elongatus CI-CVI	280 - 3000[1]	8.2 - 7.2[1]	33 d	6.9-10	↔	copepodite abundance	this study	MS
					↔	copepodite stage development		
Family Euchaetidae Giesbrecht, 1893								
Euchaeta marina	1500 - 27,000		≤ 10 d	10	↓	survival	Watanabe et al. (2006)	
Paraeuchaeta birostrata	1100 - 21,000		≤ 10 d	3	↓	survival	Watanabe et al. (2006)	
Family Heterorhabdidae Sars, 1902								
Heterostylites major	1100/8800		≤ 10 d	3	↓	survival	Watanabe et al. (2006)	

AchE: acetylcholinesterase; ATPase: adenosinetriphosphatase; GPx: glutathione peroxidase; GST: glutathione S-transferase; SOD: superoxide dismutase; GSH: reduced glutathione; GSSG: oxidized glutathione.
[1]initial pCO_2/pH at the start of the study; *significant effect, but not consistent

Table A1 (continued).

Copepod species	pCO_2 (µatm)	pH	Exposure time	Temp (°C)	Response	Reference	Remarks
Family Metridinidae Sars, 1902							
Metridia pacifica	3500 - 22,000		≤ 10 d	3	↓ survival	Watanabe et al. (2006)	
Family Temoridae Giesbrecht, 1893							
Temora longicornis NI-CVI	280 - 3000[1]	8.2 - 7.2[1]	33 d	6.9-10	↔ abundance ↔ stage development	this study	MS
Temora longicornis ♀/eggs	480/620/ 750/9830	7.97/7.85/ 7.78/6.71	4 d	14.7	↔ egg production ↔ hatching success	McConville et al. (2013)	
Epi-/meso-/bathypelagic/ eurybathic copepod assemblages	1100 - 98,000	7.65 - 6.02	≤ 10 d	3/17	↓ survival	Watanabe et al. (2006)	
Calanoid species	1000	7.72	15 d	13.7	↔ abundance	Troedsson et al. (2012)	MS
Cyclopoida							
Family Oithonidae Dana, 1853							
Oithona similis NI-CVI	280 - 3000[1]	8.2 - 7.2[1]	33 d	6.9-10	↔ abundance ↔ stage development	this study	MS
Oithona similis adults	700/1000	7.8/7.6	7 d	-1.7	↓ survival	Lewis et al. (2013)	
nauplii <60 µm (*Oithona similis*?)	700/1000	7.8/7.6	7 d	-1.7	↓ survival	Lewis et al. (2013)	
Cyclopoid species	1000	7.7	15 d	13.7	↔ abundance	Troedsson et al. (2012)	MS

[1]initial pCO_2/pH at the start of the study

Table A1 (continued).

Copepod species	pCO_2 (µatm)	pH	Exposure time	Temp (°C)	Response	Reference	Remarks
Harpacticoida							
Family Diosaccinae Sars, 1906							
Amphiascoides atopus ♀		6.67 - 5.47	96 h	25	↓ survival	Pascal et al. (2010)	
Schizopera knabeni ♀		6.44 - 5.29	96 h	25	↓ survival	Pascal et al. (2010)	
Family Ectinosomatidae Sars, 1903							
Microsetella sp. CI-CVI	280 - 3000[1]	8.2 - 7.2[1]	33 d	6.9-10	↔ abundance	this study	MS
Family Harpacticidae Dana, 1846							
Tigriopus japonicus nauplii	39,000 - 130,000	6.26 - 5.74	24 h	20	↔ survival ↔ ratio of mobile nauplii	Kita et al. (2013)	
Tigriopus japonicus NI-CVI, eggs	5800/37,000/110,000	7.11/6.31/5.85	21 d	23	↑ developmental time ↔ sex ratio ↓ hatching success ↓ nauplii survival	Kita et al. (2013)	LCS
Family Tisbidae Stebbing, 1910							
Tisbe battagliai eggs, NI-CVI	284 - 647	7.95 - 7.67	~ 60 d	19.25	↓ nauplii production ↓ growth ↑ cuticle carbon to oxygen concentration	Fitzer et al. (2012)	MGS
Tisbe sp. CI-CVI	280 - 3000[1]	8.2 - 7.2[1]	33 d	6.9-10	↔ abundance	this study	MS
bottom-dwelling harpacticoids		~ 6.9	30 d	1.6	↓ survival	Thistle et al. (2005)	*in situ* study
bottom-dwelling harpacticoids		~ 6.9	30 d	1.6	↓ survival ↔ dwelling depth	Thistle et al. (2006)	*in situ* study
bottom-dwelling harpacticoids		~ 7.6	37 d	1.58	↑ escape from seabed	Thistle et al. (2007)	*in situ* study

[1]initial pCO_2/pH at the start of the study

Table A1 (continued).

Copepod species	pCO_2 (µatm)	pH	Exposure time	Temp (°C)	Response	Reference	Remarks
bottom-dwelling harpacticoids	2000	7.4	56 d	13-17.8	↔ abundance ↔ biomass	Kurihara et al. (2007)	microcosm study
harpacticoid species	1000	7.7	15 d	13.7	↔ abundance	Troedsson et al. (2012)	MS

Eidesstattliche Erklärung

(Gem. § 6 (5) Nr. 1-3 PromO)

Hiermit versichere ich, dass ich

1.) die vorliegende Arbeit ohne unerlaubte fremde Hilfe angefertigt habe,

2.) keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe und

3.) die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Bremerhaven, den 03.02.2014

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Nicole Hildebrandt