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Zooplankton community responses to Ocean Acidification

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Credit for the pictures included in the cover and back cover is for F. Dahlke (*Acartia* sp.) and M. Algueró Muñiz (*Hybocodon prolifer*, Gran Canaria KOSMOS2014 Experiment).

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SUMMARY

Ocean acidification is affecting marine ecosystems directly through changes in pH, as well as indirectly, via trophic pathways. Thus, to evaluate impacts of ocean acidification on marine communities it is necessary to consider the potential pCO_2 effects on population dynamics as well as community trophic interactions. Within the framework of the BIOACID II project (Biological Impacts of Ocean ACIDification), the overarching goal of this thesis was to study the effects of ocean acidification on zooplankton, focusing on copepods and jellyfish. The main results are described in four chapters (CHAPTER I to IV), each of which corresponds to a manuscript.

The first part of this thesis evaluated pCO_2 effects on natural mesozooplankton communities from a boreal fjord (CHAPTER I) and the subtropical Northeast Atlantic (CHAPTER II). Large-scale pelagic mesocosm units ("Kiel Off-Shore Mesocosms for Future Ocean Simulations": KOSMOS) were artificially enriched in CO_2 to simulate future ocean conditions. In both experiments, we detected species-specific sensitivities to ocean acidification in copepods, as well as positive pCO_2 effect on total mesozooplankton abundances under high-CO_2 bloom conditions, caused by a bottom-up effect. During the Gullmar Fjord KOSMOS2013 experiment (CHAPTER I) species-specific sensitivities to CO_2 were detected in copepods, as well as in hydromedusae. However, these effects on single species were not translated into the structure or the diversity of the community, likely due to the overwhelmingly dominance of *Pseudocalanus acuspes*, which resulted to be more abundant under acidic conditions, especially the younger (copepodite) life stage. In the Gran Canaria KOSMOS2014 study (CHAPTER II) a significant effect of pCO_2 on phytoplankton succession was detected, ultimately affecting the development of the plankton community only after a simulated bloom event. The zooplankton community responded to the phytoplankton bloom in all mesocosms, although the response was delayed under high pCO_2 conditions. The most abundant mesozooplankters were calanoid copepods, which did not respond to CO_2 treatments during the pre-bloom phase of the experiment. However calanoids were more abundant under elevated pCO_2 conditions than in low- pCO_2 levels in the post-bloom phase. Bottom-up effects of CO_2-driven increases in phyto- and microzooplankton standing stocks

would explain the increase in copepod abundance during both experiments. These results suggest that, under realistic end-of-century scenarios, the above-mentioned ocean acidification effects detected on copepods could potentially affect biomass transfer to higher trophic levels.

As in community experiments it is not possible to separate out the pCO_2 direct and indirect effects, mesocosms studies were combined with laboratory experiments in the second part of this thesis work. The aim was to evaluate direct and indirect effects of global change conditions on the two main groups of interest for this thesis: copepods and jellyfish. Apart from direct acidification effects, the increasing carbon availability in the marine environment will likely change primary production and the quality of phytoplankton as food for higher trophic levels, showing higher C:nutrient ratios as CO_2 availability increases. Hence, a change in biochemical composition when culturing algae (*Rhodomonas salina*) in elevated pCO_2 levels caused a change in food quality, affecting zooplankton by decreased growth and development. Indirect negative pCO_2 effects were observed on the dinoflagellate *Oxyrrhis marina* and nauplii and copepodite stages of the copepod *Acartia tonsa*. Direct pH effects on these consumers seem to be of lesser importance than the indirect effects caused by a CO_2-associated decrease in algal quality when having only a food source (CHAPTER III), unlike the positive CO_2-effect observed in copepods when feeding on natural plankton communities. Direct pH effects on zooplankton, however, must be placed in a global change context, considering that ocean acidification in future oceans will not act alone but in combination with other climate factors such as warming and deoxygenation. The direct effects of these three stressors in conjunction were thus studied on 1-day-old ephyrae of the moon jellyfish (*Aurelia aurita*) from a North Sea subpopulation off Helgoland Island (Germany). The results obtained during this experiment point that end-of-century pCO_2 scenarios will not affect these ephyrae in a substantial way. However, *A. aurita* may not be robust to larger changes in ocean pH, warming and deoxygenation, especially if simultaneous increases in atmospheric pCO_2 levels and seawater temperature occur (CHAPTER IV). *A. aurita* is an ecologically and economically relevant species due to its interactions with commercially important fish species, hence the tolerance or resilience of this jellyfish to climate change might be detrimental for future fisheries.

Overall, this thesis showed that major components of mesozooplankton communities might be resilient, or even benefit from OA under end-of-century scenarios when grazers can compensate the deficiencies in the food quality caused by the increased CO_2 by selecting foods which most closely match their metabolic needs. Since copepods serve as major food source for fish as well as jellyfish, CO_2-driven trophic cascades as the ones described here might have important implications for future fisheries and ecosystem services. Future research should consider to focus on the effects of climate change on communities to make predictions, since the outcome based on single species experiments does not reflect the manifold and complicated interactions within communities. Thus, further long-term community studies are still necessary in order to take adaptive responses into account and discern how the responses to elevated pCO_2 described here could affect future generations in both copepod and jellyfish.

ZUSAMMENFASSUNG

Die Ozeanversauerung beeinflusst die marinen Ökosysteme direkt durch Veränderungen des pH-Werts, sowie indirekt über trophische Beziehungen. Um die Auswirkungen der Ozeanversauerung auf marine Gemeinschaften zu bewerten, ist es notwendig, die potenziellen pCO_2-Effekte auf die Populationsdynamik sowie gegenseitige trophischen Wechselwirkungen zu betrachten. Im Rahmen des BIOACID-II-Projekts (Biologische Auswirkungen der Ozeanversauerung) war das übergeordnete Ziel dieser Arbeit, die Auswirkungen der Ozeanversauerung auf Zooplankton mit Fokus auf Copepoden und Quallen zu untersuchen. Die Hauptergebnisse sind in vier Kapiteln (KAPITEL I bis IV) beschrieben, die jeweils einem Manuskript entsprechen.

Der erste Teil dieser Arbeit befasst sich mit pCO_2-Effekten auf natürliche Mesozooplankton-Gemeinschaften aus einem borealen Fjord (KAPITEL I) und dem subtropischen Nordostatlantik (KAPITEL II). Große pelagische Mesokosmen ("Kiel Off-Shore-Mesokosmen für zukünftige Ozeansimulationen": KOSMOS) wurden mit CO_2 angereichert, um zukünftige Ozeanbedingungen zu simulieren. In beiden Experimenten wurden Spezies-spezifische Sensitivitäten gegenüber Ozeanversauerung bei Copepoden nachgewiesen, sowie ein positiver pCO_2-Effekt auf die gesamte Mesozooplankton-Abundanz bedingt durch Bottom-Up Prozesse der hoch-CO_2-Blütenbedingungen. Während des Gullmarfjords KOSMOS2013-Experiment (KAPITEL I) wurden Spezies-spezifische Empfindlichkeiten gegenüber hohen pCO_2-Werten in Copepoden, sowie in Hydromedusen nachgewiesen. Allerdings fanden sich diese Effekte auf einzelne Arten nicht in der Struktur oder Vielfalt der Gemeinschaft wieder, wahrscheinlich aufgrund der überwiegenden Dominanz von *Pseudocalanus acuspes*, welcher, vor allem in der jüngeren Lebensstadien (Copepodit) höhere Abundanzen unter sauren Bedingungen erreichte. In der Gran Canaria KOSMOS2014-Studie (KAPITEL II) wurde eine signifikante Wirkung von pCO_2 auf die Phytoplankton Sukzession festgestellt, welche die Entwicklung der Plankton-Gemeinschaft nach der simulierten Blüte beeinflusste. Die Zooplankton-Gemeinschaft reagierte auf die Phytoplanktonblüte in allen Mesokosmen, jedoch war diese Reaktion unter hohen pCO_2-Bedingungen verzögert. Die häufigsten Mesozooplankter waren calanoide Copepoden, auf die die CO_2-Zugabe vor der Blütephase

keinen Einfluss hatte. Calanoide Copepoden waren allerdings unter erhöhten pCO_2-Bedingungen nach der Blütephase abundanter als bei niedrigen pCO_2-Konzentrationen. Bottom-up-Effekte durch die CO_2-bedingte Zunahmen der Phyto- und Mikrozooplankton-Bestände können eine Erklärung der Zunahme der Copepoden-Häufigkeit bei beiden Experimenten sein. Diese Ergebnisse deuten darauf hin, dass unter realistischen Szenarien wie sie Ende des Jahrhunderts erwartet werden die oben erwähnten Effekte der Ozeanversauerung auf Copepoden möglicherweise den Transfer von Biomasse auf höhere trophische Ebenen beeinflussen können.

Da es bei Experimenten welche die biologische Gemeinschaft betrachten nicht möglich ist, zwischen direkten und indirekten Effekten zu unterscheiden, wurden im zweiten Teil dieser Arbeit Mesokosmosstudien mit Laborexperimenten kombiniert. Ziel war es, direkte und indirekte Auswirkungen der globalen Veränderungen auf die beiden Hauptinteressensgruppen dieser Arbeit zu untersuchen: Copepoden und Quallen. Neben direkten Effekten der Ozeanversauerung führt die zunehmende Kohlenstoffverfügbarkeit in den marinen Lebensräumen zu Änderungen der Primärproduktion und beeinflusst somit die Qualität von Phytoplankton (höhere C:Nährstoff Verhältnisse unter erhöhten CO_2-Bedingungen) als Nahrung für höhere Trophiebenenen. Daher führte eine Veränderung der biochemischen Zusammensetzung von Algen (*Rhodomonas salina*) die unter erhöhten pCO_2-Konzentrationen kultiviert wurden zu einer Veränderung der Nahrungsqualität, welche Wachstum und Entwicklung des Zooplanktons negativ beeinflusste. Indirekte negative pCO_2-Effekte wurden für den Dinoflagellat *Oxyrrhis marina* sowie für Nauplien und Copepodit-Stadien des Copepoden *Acartia tonsa* beobachtet. Direkte pH-Effekte auf diese Konsumenten scheinen von geringerer Bedeutung zu sein als indirekte Effekte durch die CO_2-bedingte Abnahme der Algenqualität wenn nur eine Nahrungsquelle (KAPITEL III) zu Verfügung steht, im Gegensatz zu dem beobachtetem positiven CO_2-Effekt auf Copepoden welchen eine natürliche Planktongemeinschaft als Futterquelle zu Verfügung steht. Direkte pH-Effekte auf Zooplankton müssen jedoch in einem globalen Kontext bewertet werden, da Ozeanversauerung in Zukunft nicht als einzelner Faktor sondern in Kombination mit weiteren klimatisch bedingten Faktoren wie Erwärmung und Desoxygenierung auftritt. Die direkten Effekte dieser drei Stressoren gemeinsam wurden an ein-Tag alten Ephyra-Larven der Ohrenqualle (*Aurelia aurita*) aus einer Nordsee-Subpopulation von Helgoland (Deutschland)

untersucht. Die Ergebnisse, die während dieses Experiments erzielt wurden, zeigen, dass pCO_2-Szenarien die für das Ende des Jahrhunderts erwartet werden Ephyra-Larven nicht wesentlich beeinflussen. Allerdings ist *A. aurita* möglicherweise nicht robust gegenüber größeren Veränderungen des Ozean pH-Wertes, der Erwärmung und Desoxygenierung, vor allem, wenn gleichzeitige Erhöhungen des atmosphärischen pCO_2-Gehaltes und der Meerwassertemperatur auftreten (KAPITEL IV). *A. aurita* ist aufgrund ihrer Wechselwirkungen mit kommerziell wichtigen Fischarten eine ökologisch und ökonomisch relevante Art, daher kann die Toleranz oder Widerstandsfähigkeit dieser Quallen gegenüber dem Klimawandel nachteilige Auswirkungen auf die Fischerei haben.

Insgesamt zeigte die vorliegende Arbeit, dass wichtige Bestandteile der Mesozooplankton-Gemeinschaften resilient gegenüber der am Ende des Jahrhunderts erwarteten Ozeanversauerungsbedingungen sind, oder sogar von diesen Bedingungen profitieren können, wenn sie CO_2 bedingte Mängel in der Nahrungsqualität durch eine selektive Aufnahme geeigneter Nahrung entsprechend ihren Bedürfnissen kompensieren. Da Copepoden als Hauptfutterquelle für Fische und Quallen dienen, können CO_2-getriebene trophische Kaskaden, wie sie hier beschrieben wurden, wichtige Auswirkungen auf zukünftige Fischerei- und Ökosystemleistungen haben. Die zukünftige Forschung sollte sich daher auf Auswirkungen des Klimawandels auf ökologische Gemeinschaften konzentrieren, da die Ergebnisse auf der Grundlage einzelner Artenexperimente nicht die vielfältigen und komplizierten Wechselwirkungen innerhalb der Gemeinschaften widerspiegeln. Es sind somit noch weitere langfristig angelegte Studien an marinen Gemeinschaften nötig, die auch adaptive Prozesse berücksichtigen um erkennen zu können, wie sich die hier beschriebenen Reaktionen auf erhöhte pCO_2-Werte auf künftige Generationen sowohl in Copepoden als auch in Quallen auswirken.

LIST OF ABBREVIATIONS

°C	Degree Celsius
AIC	Akaike Information Criterion
ANOSIM	Analysis of Similarity
ANOVA	Analysis of Variance
AR	Assessment Report
AWI	Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung
BAH	Biologische Anstalt Helgoland
BIOACID	Biological Impacts of Ocean Acidification
C	Carbon
C1-C6	Copepodite developmental stages
$CaCO_3$	Calcium carbonate
Chl*a*	Chlorophyll *a*
CO_2	Carbon dioxide
CTD	Conductivity, Temperature, Depth Sonde
D	Simpson's Diversity Index
DW	Deep water
ESD	Equivalent spherical diameter
F, ♀	Female
GAMM	Generalized Additive Mixed Models
GEOMAR	GEOMAR, Helmholtz Centre for Ocean Research Kiel
GHG	Greenhouse gas
GLM	Generalized Linear Models
GLMM	Generalised Linear Mixed Models
H	Hydrogen
IPCC	Intergovernmental Panel on Climate Change

IWS	Integrative water sampler
KOSMOS	Kiel Off-Shore Mesocosms for Future Ocean Simulations
M, ♂	Male
MesoZP	Mesozooplankton
MicroZP	Microzooplankton
N	Nitrogen
n	Number of individuals, size of the sample
NMDS	Non-metric Multidimensional Scaling
NO_2^-	Nitrite ion
NO_3^-	Nitrate ion
NO_x	Nitrogen oxides
O_2	Oxygen
OA	Ocean acidification
P	Phosphorus
p	p-value, statistical significance
pCO_2	Carbon dioxide partial pressure
POLMAR	Helmholtz Graduate School for Polar and Marine Research
ppm	Parts per million
RCPs	Representative Concentration Pathways
Rho	*Rhodomonas salina*
RV	Research vessel
sp., spp.	Species (sing., pl.)
µatm	Microatmosphere

1. INTRODUCTION

1.1 Ocean acidification within a global change context

Global change is being forced by human activities, the most significant driver of warming and greenhouse gases emissions since the mid-20th century (IPCC 2013). The Fifth Assessment Report (AR5) of the Intergovernmental Panel on Climate Change (IPCC) describes a set of scenarios, known as Representative Concentration Pathways (RCPs) that account for a wide range of possible changes in future anthropogenic greenhouse gas emissions (GHGs). RCPs consider a broad range of climate outcomes, from a desirable decline (RCP2.6) to a continuing rise in the emissions during the 21st century (RCP8.5) that would bring current atmospheric pCO_2 values (ca. 400 µatm) to levels of up to 1000 µatm in less than 100 years (RCP8.5 IPCC 2013).

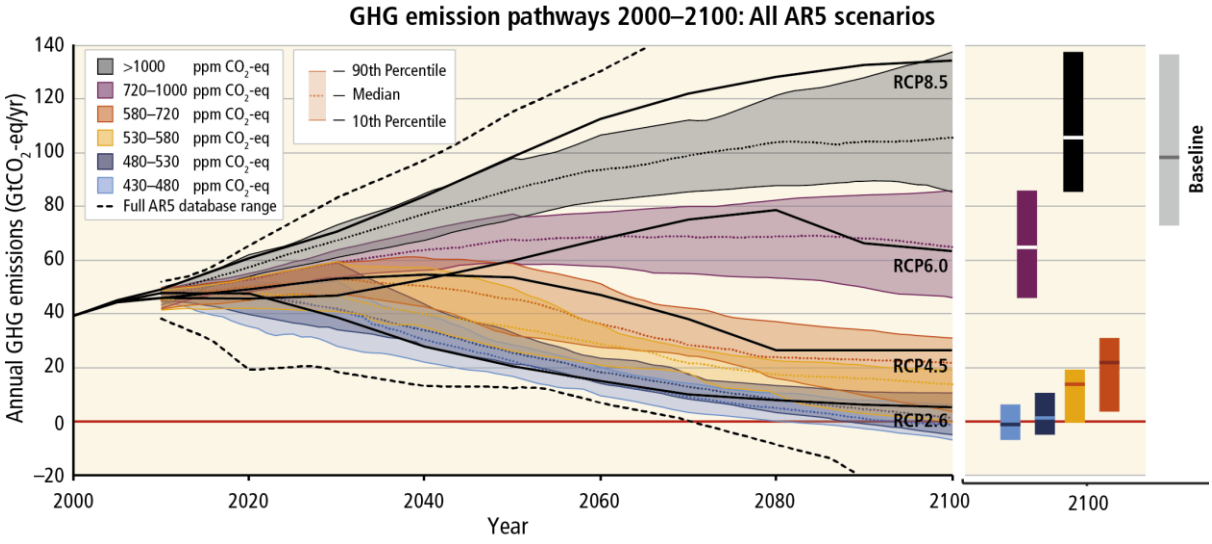

Fig 1.1: GHG Emissions Pathways (GtCO2eq/yr) in baseline and mitigation scenarios of all IPCC AR5 scenarios (including the RCPs) for different end-of-century concentration levels. Source: AR5 Synthesis Report, IPCC 2013

The uptake of atmospheric CO_2 by the ocean results in ocean acidification (OA), which can interact with other natural and anthropogenic environmental stressors such as warming (Hoegh-Guldberg et al. 2007) and deoxygenation (Melzner et al. 2013), as described below.

1.1.1 Acidification

Approximately one-third of the anthropogenic CO_2 has been taken up by the oceans (Sabine et al. 2004) leading to a reduction in pH —hence the term "ocean acidification" (Wolf-Gladrow et al. 1999; Caldeira and Wickett 2003)— and pronounced shifts in seawater carbonate chemistry occurring at a pace unprecedented in recent geological history (Doney et al. 2009).

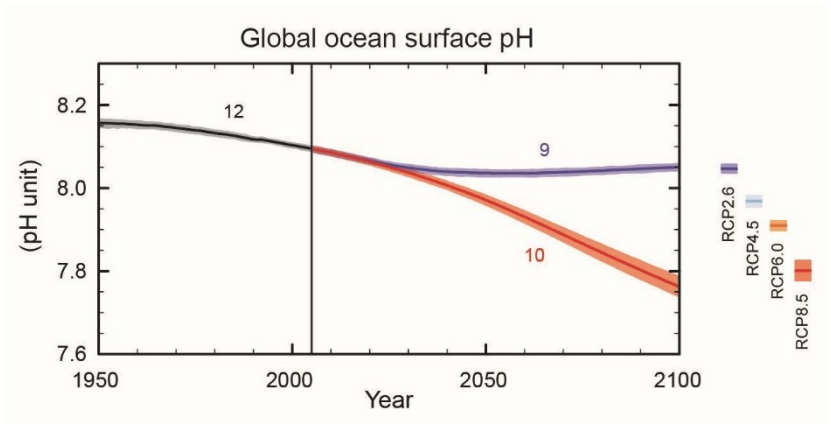

Fig 1.2: Global ocean surface pH. Simulated time series from 1950 to 2100 for global mean ocean surface pH. Time series of projections and a measure of uncertainty (shading) are shown for scenarios RCP2.6 (best case, in blue) and RCP8.5 ("business as usual", in red). Black (grey shading) is the modelled historical evolution using historical reconstructed forcings. The numbers indicate the number of models used in each ensemble. Right side of the figure: baselines for the different scenarios. Source: AR5 WG1, IPCC 2013

Seawater carbonate chemistry is governed by a series of chemical reactions:

$$CO_{2(atmos)} \rightleftharpoons CO_{2(aq)} + H_2O \rightleftharpoons H_2CO_3 \quad (1)$$

$$H_2CO_3 + H_2O \rightleftharpoons H_3O^+ + HCO_3^- \quad (2)$$

Adding CO_2 to seawater increases aqueous CO_2 ($CO_{2(aq)}$), bicarbonate (HCO_3^-), and hydronium (H_3O^+) concentrations, and the latter lowers pH according to $pH = -\log_{10}[H_3O^+]$. The continuous uptake of CO_2 from the atmosphere diminishes the buffer capacity of the seawater. The dissolution of atmospheric CO_2 in the oceans leads to increasing amounts of H_3O^+, H_2CO_3 and HCO_3^-, while the concentration of CO_3^{2-} decreases (Raven et al. 2005). Carbonic acid in its original form (H_2CO_3) is present in seawater in very low concentrations

compared to dissolved CO_2 and HCO_3^- (2). The three dissolved inorganic carbon species in seawater (CO_2, HCO_3^-, and CO_3^{2-}) are in chemical equilibrium on time scales shorter than a few minutes

$$CO_2 + 2\,H_2O \rightleftharpoons HCO_3^- + H_3O^+ \quad (3)$$

$$HCO_3^- + H_2O \rightleftharpoons CO_3^{2-} + H_3O^+ \quad (4)$$

These abiotic changes may cause direct as well as indirect effects on marine organisms, as described in 1.2.1 section.

1.1.2 Warming

Anthropogenic activities are the principal source of the observed increased rate in warming since the mid-20th century, causing ongoing biological change in marine ecosystems (Perry et al. 2005; Rosenzweig et al. 2008). The global ocean is expected to continue to warm during the 21st century, and heat will penetrate from surface to the deep ocean, affecting ocean circulation. While global average Earth surface temperature might increase up to 4°C by 2100 (RCP8.5), estimates for ocean warming in the first 100 meters are between 0.6 (RCP2.6) and 2°C (RCP8.5)(IPCC 2013).

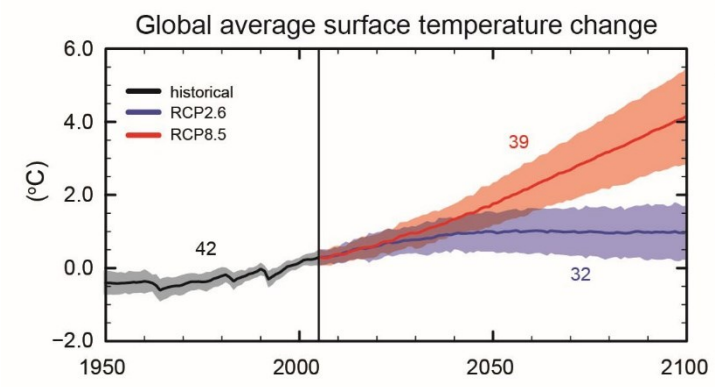

Fig 1.3: Global average surface temperature change from 1950 to 2100. Time series of projections and a measure of uncertainty (shading) are shown for scenarios RCP2.6 (best case, in blue) and RCP8.5 ("business as usual", in red). Black (grey shading) is the modelled historical evolution using historical reconstructed forcings. The numbers indicate the number of models used in each ensemble. Source: AR5 WG1, IPCC 2013

Apart from direct effects on physiological processes, ocean warming can affect individuals through e.g. increased consumption rates and accelerated development and growth (Sanford 1999), as well as reduction in organisms body size (Daufresne et al. 2009; Garzke et al. 2015; Garzke et al. 2016). This may lead to changes in community composition and phenology by earlier peak occurrences (Edwards and Richardson 2004), causing a mismatch between trophic levels and functional groups, and the consequent changes in community structure and ultimately in entire ecosystems.

1.1.3 Deoxygenation

The reduction of O_2 supply to the ocean interior responds to the warming of surface waters, which become less dense —O_2 is less soluble at warmer temperatures—, leading to a more stratified water column and reduced mixing processes (Sarmiento et al. 1998; Bopp et al. 2002; Keeling and Garcia 2002; Keeling et al. 2010). The distribution of O_2 in the ocean interior is controlled by the interplay between air-sea exchange, circulation, and biological processes (Keeling et al. 2010). Oxygen deficient conditions frequently occur in coastal waters and estuaries where high rates of photosynthetic production and the consequent eutrophication occur, fuelled by riverine runoff of fertilizers and the burning of fossil fuels (Diaz and Rosenberg 2008). This leads to high rates of O_2 consumption in subsurface waters and sediments, resulting in an accumulation of particulate organic matter, which in turn encourages microbial activity and the consumption of dissolved oxygen in bottom waters (Diaz and Rosenberg 2008; Keeling et al. 2010). Stratification may cause a reduction in (1) the supply of nutrients from subsurface to surface waters and (2) the exchange of surface and subsurface water. The former (1) would increase the production and export of organic carbon and subsurface oxygen utilization rates, causing an increase in subsurface O_2 levels. And (2) would reduce the transport of O_2 into the ocean interior, leading to an overall (1+2) decrease in interior ocean O_2 since the effect on ventilation exceeds the effect on utilization (Keeling et al. 2010). Due to the combined effects of coastal eutrophication and ocean warming (Fig 1.3), the deoxygenation trend is forecasted to continue with reductions in mean dissolved

oxygen (DO) concentrations from 1.5 to 4% (0.08 to 0.208mg O_2 L^{-1}) in 2090s relative to 1990s for all RCP scenarios (IPCC 2013).

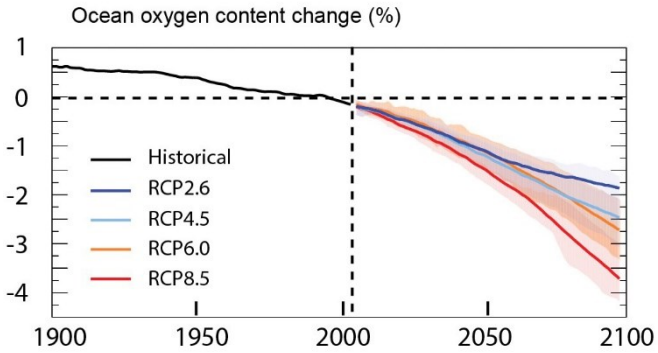

Fig 1.4: Ocean oxygen content change (1900-2100), in percentage. Time series of projections and a measure of uncertainty (shading) for different scenarios, from RCP2.6 (best case) to RCP8.5 ("business as usual"). Blackline represents the modelled historical evolution using historical reconstructed forcings. Source: AR5 WG1, IPCC 2013

The loss of DO in the world's ocean might have implications for ocean productivity and nutrient and carbon cycling in marine habitats, having significant ecosystem-level consequences (Gilly et al. 2013). In addition to lower DO solubility, warmer temperatures in combination with coastal eutrophication may increase metabolic rates and, in turn, oxygen consumption. Hypoxia thresholds vary considerably across marine organisms, although there is a conventional definition of 2mg O_2 L^{-1} to designate waters as hypoxic. However, this concentration seem to be below the empirical sub-lethal and lethal O_2 thresholds for many species, which implies that the future extent of hypoxia impacts on marine ecosystems have been generally underestimated (Vaquer-Sunyer and Duarte 2008).

1.1.4 Multiple environmental stressors

Climatic stressors do not act alone but additively, synergistically or antagonistically (IPCC 2013; Pörtner et al. 2014). Hence, OA occurs concomitantly with other global environmental factors, such as warming, deoxygenation, and increased stratification, which in turn alters salinity, the availability of nutrients and light. At the regional scale, other factors to consider

in the interactions include eutrophication, overfishing and species invasion and extinction (Riebesell and Gattuso 2015). The conjunction of these factors will determine organisms' sensitivity, modifying the windows of tolerance to the different stressors (Pörtner and Farrell 2008).

While temperature is a key climate driver for biological changes, OA modulates organisms responses to temperature (Pörtner 2008). But the effects of both stressors acting simultaneously have been reported as both synergistic and antagonistic. In the case of calanoid copepods, the effects of warming and OA have been described as antagonistic, since high-temperature can negatively affect copepod size and abundance (Garzke et al. 2015), while acidification partially compensates for the temperature effect (Garzke et al. 2016). However, same stressors have been shown to cause a synergistic effect on Atlantic cod by increasing thermal sensitivity of embryos under future pCO_2 scenarios (Dahlke et al. 2016).

Responses of marine organisms has been mostly studied by the solely effects of hypoxia (Vaquer-Sunyer and Duarte 2008) or acidification (Doney et al. 2009), and rarely both at once (Melzner et al. 2013; Steckbauer et al. 2015), although hypoxia and high-pCO_2 are expected to occur simultaneously in nature (Brewer and Peltzer 2009; Mayol et al. 2012). For example, cold low-oxygen waters are naturally supersaturated in CO_2 in coastal upwelling systems, where the combination of hypoxia and high pCO_2 have been shown to have additive effects on benthic invertebrates, reducing their respiration rates significantly (Steckbauer et al. 2015).

Thus, factors like warming, eutrophication or hypoxia have to be taken into account as they might lead to an intensification or dampening of the effects of OA (Pörtner 2008; Rost et al. 2008). Hence, there is an urgent need to use multiple-stressor approaches in climate change research in order to make solid predictions for the future.

1.2 Ocean acidification implications: from individuals to ecosystems

Responses to OA at organism level may not reflect those at the community and ecosystem level, since biotic interactions may lead to a dampening or amplification of OA effects on

single species (Rossoll et al. 2013). Therefore, elucidating an organism's response to changing ocean conditions must be integrated in community studies that consider competitive and trophic interaction effects (Riebesell and Gattuso 2015). To this end, plankton community experiments such mesocosms (Riebesell et al. 2013) allow to study organisms' responses within a more realistic context, and can be combined with laboratory studies for a better understanding of the physiological mechanisms that explain the individual tolerance or sensitivity to OA.

Within marine ecosystems, zooplankton is a key component along with phytoplankton, forming the base of most marine food webs. There is a strong size structure within the plankton community, which in turn comprises organisms that spend their whole life in the water column (holoplankton) as well as others whose life cycle includes planktonic and benthic phases (meroplankton). In this thesis I considered zooplankton size categories including microzooplankton (20-200 µm) and mesozooplankton (0.2-20 mm).

1.2.1 Direct and indirect pCO_2 effects

Increase in atmospheric CO_2 and the consequent OA may affect marine organisms either directly (i.e. by changes in pH or carbon availability) or indirectly (via trophic pathways). Direct effects may impact zooplankton through the acidification of body fluids (also known as hypercapnia), by changing intracellular pH, membrane potentials and enzymatic activities (Fabry et al. 2008; Nielsen et al. 2010). When CO_2 levels increase in seawater, dissolved CO_2 diffuses more easily across body surfaces to equilibrate CO_2 concentrations in both intra- and extracellular spaces. This CO_2 can interact with internal body fluids causing internal pH to decrease. Generally, marine invertebrates seem to be especially sensitive to high levels of hypercapnia (Melzner et al. 2009), which can cause the suppression of metabolic processes (Michaelidis et al. 2005; Pörtner 2008) and disrupt acid-base homeostasis (Miles et al. 2007). The ability of marine calcifiers (pteropod molluscs, foraminifera, and some benthic invertebrates) to produce calcareous skeletal structures ($CaCO_3$) is directly affected by seawater CO_2 chemistry (e.g. Fitzer et al. 2014; Riebesell et al. 2017). Accordingly, the effects of chronic exposure to elevated pCO_2 on calcifiers and the long-term implications of reduced

calcification rates could compromise the fitness of these organisms and shift the competitive advantage towards non-calcifiers (Fabry et al. 2008)

Indirect OA effects are induced by changing composition of prey communities or by changes in the biochemical content of prey, which may alter the responses of consumers (Rossoll et al. 2012; Schoo et al. 2013). Increased pCO_2 can stimulate carbon fixation by primary producers and thereby reduce the nutrient content relative to carbon (Urabe et al. 2003; Riebesell and Tortell 2011). Thus C:N and C:P ratios in marine systems can be expected to increase as a direct OA effect, having direct consequences for the phytoplankton community by altering their own stoichiometry (van de Waal et al. 2010) and therefore determining the food quality for primary (Boersma et al. 2009) and secondary consumers (Lesniowski et al. 2015). Despite the fact that herbivores buffer much of the variance in nutrient stoichiometry of their food and do not transfer it to higher trophic levels, effects of growing conditions of the primary producers can travel up the trophic web (Boersma et al. 2008). In laboratory experiments, it has been observed that *Rhodomonas salina* grown under elevated pCO_2 (800 ppm) had a higher C:nutrients ratio which in turn affected adversely *Acartia tonsa* copepodites' development (Schoo et al. 2013). In similar bi-trophic experiment (*A. tonsa* feeding on *Thalassiosira pseudomana*), Rossoll et al. (2012) demonstrated how ocean acidification-induced food quality deterioration may constrain trophic transfer, resulting in a decrease in copepod somatic growth and egg production.

1.2.2 OA effects on individuals: copepods and jellyfish

Throughout this thesis, single species focus has been on copepods and jellyfish (hydromedusae and scyphomedusae) which are main components in marine food webs. Copepods represent the link between phytoplankton and planktivorous predators since they can graze on primary producers and microzooplankton forms (Atkinson 1996; Calbet and Alcaraz 2007) and are an important food source for higher trophic levels such as fish and jellyfish (Turner 2004). In turn, jellyfish may not only compete with fish for food resources, but also predate on fish eggs and larvae (Purcell and Arai 2001). By using these two taxa, OA effects could be studied in two different trophic levels, from omnivorous copepods

(secondary consumers) to medusae (tertiary consumers), allowing us a better understanding of OA effects on food webs.

- Copepods

Copepods, which form a subclass within the subphylum Crustacea, are probably the most abundant metazoans on Earth (Mauchline 1998). They are globally distributed, both in freshwater and marine environments, and are a key component in the planktonic food webs (Mauchline 1998). Copepods are important prey of fish larvae and other planktivores. Small planktonic copepods exhibit a variety of reproductive strategies to compensate for population decrease due to predation, including having high fecundity and growth rates when not limited by insufficient food (Turner 2004). Some copepod species are broadcast spawners, while others carry their eggs in an egg sack attached to the female genital opening. A major factor affecting the timing and magnitude of spawning of calanoid copepods is the energy supply for gonad development, so their reproductive strategies are reflected by the female gonad adaptations to specific environmental conditions (Niehoff 2007). Concerning their life cycle, copepods are holoplanktonic, and their developmental stages include six naupliar stages (N1-N6) and six copepodite stages (C1-C6) before reaching the adult stage.

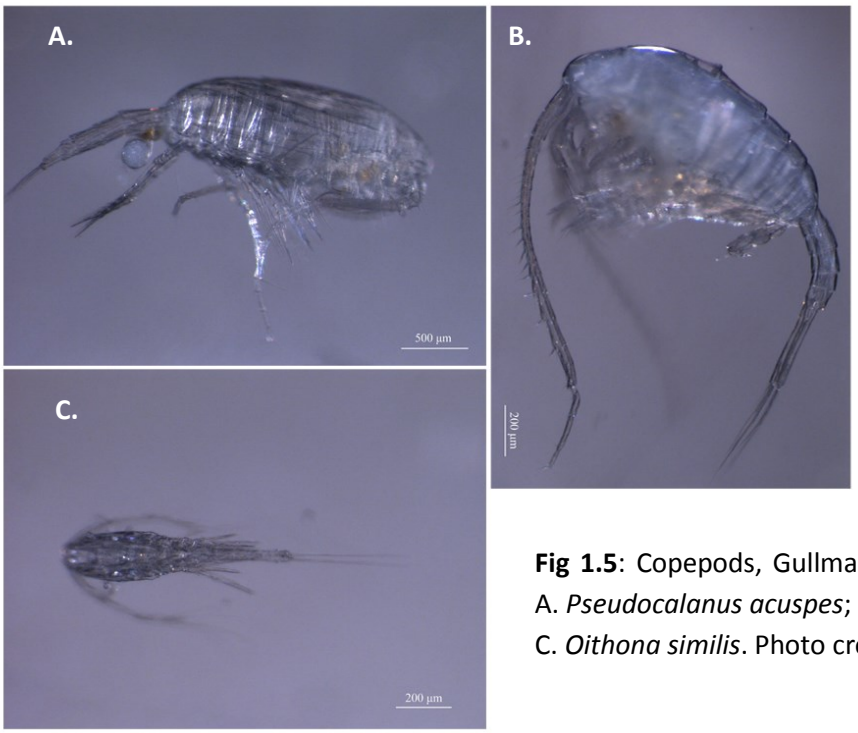

Fig 1.5: Copepods, Gullmar Fjord KOSMOS2013. A. *Pseudocalanus acuspes*; B. *Temora longicornis*; C. *Oithona similis*. Photo credit: R. Schüller.

Copepods' sensitivity to OA varies among different species and even between life stages within species (Isari et al. 2015b). For example, it has been shown that early life stages are likely to be the most sensitive to increased pCO_2 levels, resulting in a potential negative effect on survival and/or development (e.g. Cripps et al. 2014a; Pedersen et al. 2014b). Additionally, different sensitivities to OA have been related to copepod habitats, with species more regularly exposed to natural pH fluctuations (as vertical migrators or coastal species) being the most tolerant to OA (Lewis et al. 2013; Almén et al. 2014). Diverse copepod responses to OA effects have been also reported depending on the length of the exposure and the pCO_2 levels (see Isari et al. 2015b and the references therein). Yet, several calanoid species have demonstrated a high resilience in fitness at realistic end-of-century scenarios (~1000 ppm pCO_2) (e.g. Zhang et al. 2011; Weydmann et al. 2012; McConville et al. 2013). The potential indirect effects of OA (i.e. induced by changes in nutritional quality of preys) may also be determinant to understand pCO_2 effects on copepods and marine food webs (Rossoll et al. 2012; Schoo et al. 2013; Isari et al. 2015a). Most of these experiments, however, are based on short-term pCO_2 exposures, which may only indicate an initial, acute, response to OA. These short-term detrimental effects are susceptible to be lessened by homeostatic mechanisms, while transgenerational effects can buffer pCO_2 effects, giving thus time for genetic adaptation (Thor and Dupont 2015; Vehmaa et al. 2016). Hence, there is a general call for multigenerational studies that consider direct as well as indirect effects of prolonged exposure times under end-of-century pCO_2 scenarios that allow us to take adaptive responses into account.

- Jellyfish

The term *jellyfish* includes a wide and diverse group of gelatinous species classified in the phyla Cnidaria and Ctenophora. This thesis is focused on planktonic forms of the class Scyphozoa and the class Hydrozoa, within the phylum Cnidaria (hereafter referred to as jellyfish). Most scyphozoan species (e.g. *Aurelia aurita*) have metagenetic life cycles, including the planktonic medusae and the benthic long-living bottom-dwelling polyps. Seasonal polyp strobilation lead to the release of ephyrae, which in turn develop into large and conspicuous adult medusa (see Fig 1.6). Scyphomedusae feed on zooplankton, and may also predate on fish eggs and fish larvae. Some hydromedusae are holoplanktonic although most have a life cycle similar to scyphomedusae, where the medusa phase is usually small (<1 cm) and

inconspicuous (Fig 1.7), and the benthic -often colonial- polyps are called hydroids. Essentially carnivorous, some hydromedusae may feed on bacteria, protozoans, phytoplankton, and even dissolved organic matter (Bouillon et al. 2006).

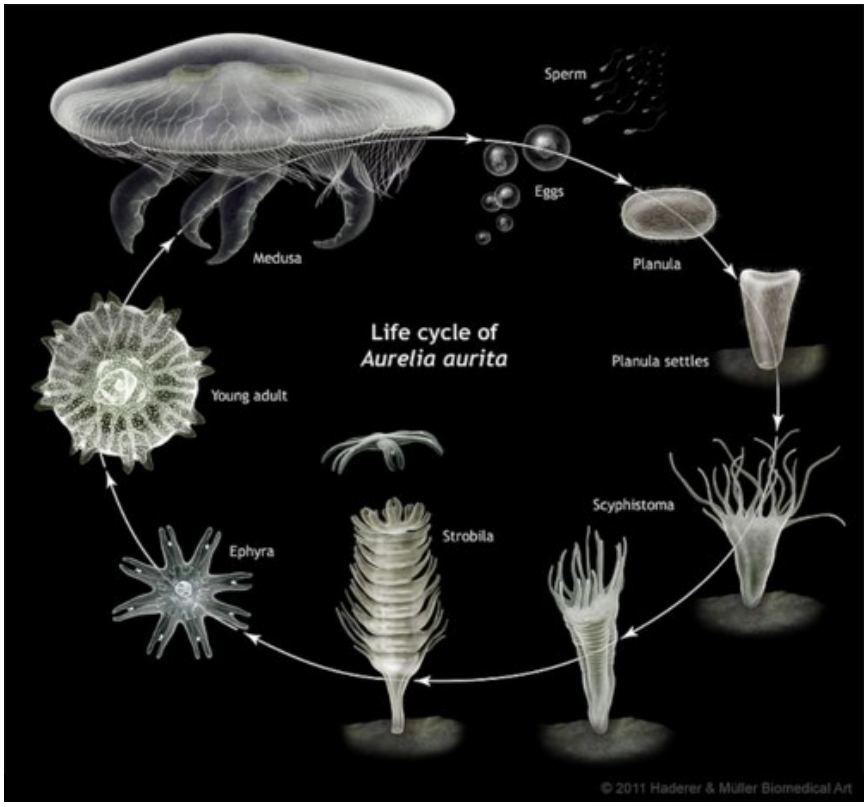

Fig 1.6: Life cycle of the moon jellyfish, *Aurelia aurita*. Fertilised eggs turn into a planulae, which settle down in hard surfaces and originates a scyphistoma or polyp. When the polyp strobilates, it releases hundreds of ephyrae, which in turn develop into adults. Image credit: © 2011 Haderer & Muller Biomedical Art.

The ability of jellyfish to occur in large numbers (i.e. to bloom) is due to the existence of both asexual (polyp) and sexual stages (medusa) in their life cycle (Purcell et al. 2007). These blooms, however, seem to coincide with human proliferations and environmental perturbations (Purcell 2012). During recent years, several studies have linked climate variation and global gelatinous zooplankton blooms (Lynam et al. 2004; Purcell 2005), because of the purported tolerance of jellyfish to human-driven ecosystem changes (Purcell 2012). That tolerance to environmental stressors suggest that jellyfish may take advantage of the vacant niches made available by the negative effects of climate change on other taxa such as fish (e.g. Hays et al. 2005; Purcell et al. 2007; Purcell 2012). Accordingly, there is evidence of inverse correlations between biomasses of jellyfish and fish, probably because of reduced

competition for zooplankton when forage fish are depleted (Daskalov et al. 2007; Purcell 2012). In this sense, overfishing is an additional key environmental driver that could positively affect jellyfish due to the removal of competitors and predators (Purcell and Arai 2001; Arai 2005).

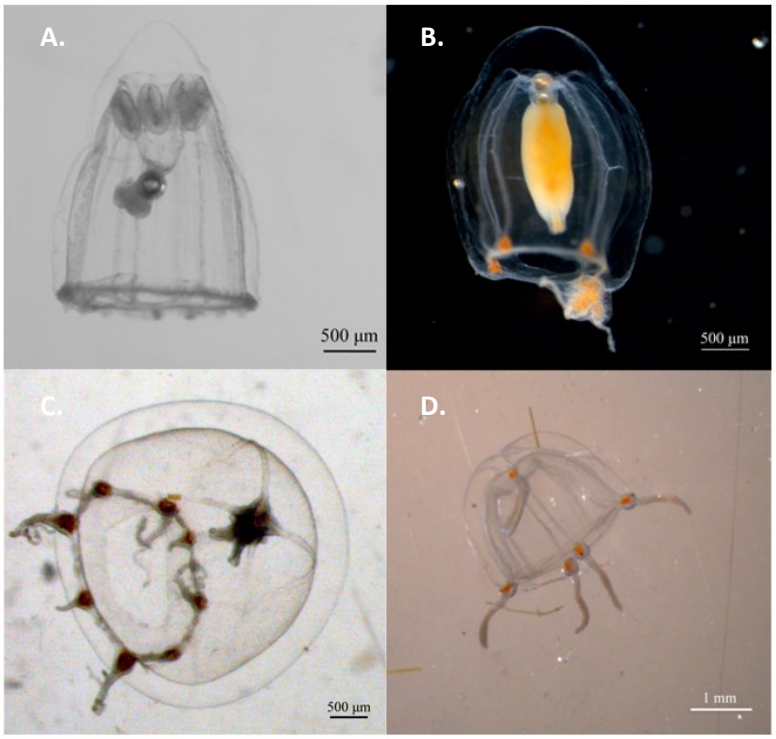

Fig 1.7: Hydromedusae, Gullmar Fjord KOSMOS2013. A. *Aglantha digitale*, B. *Hybocodon prolifer*, C. *Rathkea octopunctata*, D. *Sarsia tubulosa*. Photo credit: M. Algueró-Muñiz.

Based on long-term datasets, no solid relationships between jellyfish abundance and OA have been found to date (Attrill et al. 2007; Richardson and Gibbons 2008), but data about potential effects of changing carbonate chemistry conditions on this group is still scarce. Since most scyphomedusae and some hydromedusae such as *Aglantha digitale* possess statoliths (which are calcium-based structures functioning in equilibrium reception) medusae could be target organisms for direct pH decrease effects. One of the only studies testing the effects of diminished pH on scyphozoans reported a high tolerance of the scyphomedusa *Aurelia* sp. to OA and no effect of lower pH on the number of statoliths but a reduction on their size (Winans and Purcell 2010). Such a reduction could potentially affect orientation and swimming activities of the free-swimming stages (ephyrae and medusae). Furthermore, in scyphozoans, the size of the medusa population largely depends on the recruitment, reproduction and survival of the early life stages (Fu et al. 2014). Thus, the latter must be considered as the bottleneck of medusae proliferations and target organisms for climate change experiments.

1.2.3 OA effects on plankton communities

One of the key gaps in the current knowledge on OA effects regards the impact of increased-pCO_2 on ecological interactions within the complexity of natural ecosystems (Pörtner and Farrell 2008; Harley 2011). For example, pH variation in coastal environments under OA is influenced by biotic parameters such as photosynthesis and respiration, which also vary depending on biotic and abiotic factors (Dupont and Pörtner 2013).

The response of whole communities to increased pCO_2 has proven difficulties to assess and consequently has been studied to a far lesser extent than bi- or tri-trophic laboratory experiments. Previous mesocosms studies on natural coastal plankton communities from Norwegian fjords (Suffrian et al. 2008; Calbet et al. 2014; Hildebrandt et al. 2016), the Arctic (Aberle et al. 2013; Niehoff et al. 2013) and the Baltic Sea (Horn et al. 2016a; Lischka et al. 2017) mostly reported on a tolerance of zooplankton towards high CO_2 concentrations, or only subtle changes in the zooplankton community. Focusing on the outdoor mesocosms (Kongsfjorden, Svalbard 2010 (Schulz et al. 2013), Raunefjord, Norway 2011 (Endres et al. 2014) and Tvärminne Storfjärden, Finland 2012 (Paul et al. 2016)), the effects of high pCO_2 seemed to affect the microzooplankton rather than higher trophic levels. Thus, no significant pCO_2 effects were described on copepods, except for a reduction in adult females size under high-pCO_2 conditions (Hildebrandt et al. 2016; Vehmaa et al. 2016) and overall, no pCO_2 effect was observed on the abundances and structure of Arctic mesozooplankton communities (Niehoff et al. 2013; Lischka et al. 2015; Lischka et al. 2017). However, Lischka et al. (2015) described significant changes in microzooplankton community composition, with a shift towards smaller species/genus with increasing pCO_2 levels. This reduction in size might respond to the increased dominance of smaller-sized phytoplankton (picophytoplankton <3µm) previously reported in high pCO_2 treatments in all mesocosms experiments (Brussaard et al. 2013; Bermúdez et al. 2016; Crawfurd et al. 2016). Autotrophic standing stocks (chlorophyll *a*) were consistently higher at high pCO_2 (see (Alvarez-Fernandez et al. submitted)), and zooplankton responses —if existing— were detected after the phytoplankton blooms (Lischka et al. 2017) when the consequent nutrient depletion occur.

Overall, OA effects in plankton communities seem to be related to nutrients availability, being more intense at limiting inorganic nutrient concentrations (Paul et al. 2015; Sala et al. 2015; Alvarez-Fernandez et al. submitted). Hence, coastal marine systems are likely to be more

resilient than others to OA, as nutrients are generally replete and the natural CO_2 fluctuation in these areas is already substantial (Hoegh-Guldberg and Bruno 2010; IPCC 2013), although studies in oligotrophic waters are still scarce to date. As previous mesocosms studies on coastal areas lasted for relatively short periods of time (30 (Schulz et al. 2013), 34 (Endres et al. 2014) and 45 days (Paul et al. 2016), respectively), there is a call for long-term OA studies to uncover OA-sensitive stages of plankton succession (Bach et al. 2016b), as well as for studies that allow the comparison between nutrient-replete and nutrient-deplete systems to assess the impact of OA in plankton communities.

2. AIMS & OUTLINE

Anthropogenic activities are forcing climate to change in an unprecedented pace, hence affecting marine ecosystems under a simultaneous combination of environmental stressors. Among these, I focused my study on ocean acidification (OA), which is a consequence of the increasing trend in atmospheric pCO_2 levels. Ocean is absorbing about one third of that pCO_2 (Sabine et al. 2004), consequently causing a decrease in sea water pH and changes in carbonate chemistry. While nowadays OA effects on primary producers (Bach et al. 2016b; Eberlein et al. 2017) and calcifying organisms (Lischka et al. 2011; Riebesell et al. 2017) seem to be better understood, there is still a lack of knowledge about OA effects on secondary consumers in a community context.

The aim of this thesis was to analyse how mesozooplankton from different ecosystems is affected by OA, as well as the link between individual and community responses to increased pCO_2 levels. Accordingly, mesocosms studies in natural plankton communities were combined with laboratory experiments aiming for a deeper understanding of the potential pCO_2 effects on the ecophysiology of mesozooplankton. The aim of the first mesocosms study (Gullmar Fjord KOSMOS 2013) was to analyse the influence of realistic end-of-the-century OA scenarios on a natural winter-to-summer plankton succession in a coastal pelagic ecosystem (Bach et al. 2016b). The second mesocosms study (Gran Canaria KOSMOS2014) was focused on the effect of elevated pCO_2 levels on plankton community, with a particular focus on possible differences between oligotrophic conditions and periods of high productivity in response to the simulated upwelling of deep water (Taucher et al. 2017a). Zooplankton community responses to OA were thus studied in two different latitudes (boreal, subtropical) and nutrient regimes (eutrophic, oligotrophic). Effects to consider could be either (a) direct, by e.g. changes in physiology and metabolism associated with increases in CO_2 and/or decreases in pH, or (b) of indirect nature, for example based on altered elemental and biochemical composition of autotroph production and trophic interactions.

The main objectives of this thesis are:

• to analyse the effect of end-of-century pCO_2 levels on eutrophic and oligotrophic mesozooplankton communities structure (abundance, biomass and taxonomic composition) during the mesocosms experiments,

• to determine trophic interactions and grazing impacts of mesozooplankton on microzooplankton and phytoplankton standing stocks,

• to study the metabolic and physiological condition of copepod and jellyfish key species under different pCO_2,

• to determine direct and indirect pCO_2 effects on mesozooplankton growth and development,

• to analyse the direct effect of pCO_2 in conjunction with other climatic stressors on scyphomedusae physiology, and finally

• to study the link between individual and community responses to increased pCO_2 levels.

3. METHODOLOGICAL CONSIDERATIONS

Mesocosms setup and CO_2 manipulation

Experimental units during GullmarFjord KOSMOS2013 (CHAPTER I) and Gran Canaria KOSMOS2014 experiments (CHAPTER II) consisted in mesocosms (Kiel Off-Shore Mesocosms for Future Ocean Simulations, KOSMOS (Riebesell et al. 2013)). Each unit comprised a 8 m floatation frame, a thermoplastic polyurethane foil mesocosm bag (1 mm thick) that allowed for light penetration in the PAR spectrum, a 2 m long conical sediment trap with a pump system, a dome-shaped hood on top of the floatation frame, weights at the bottom of the floatation frame and the lower end of the bags to maintain an upright position when exposed to wind and wave activity, and various ropes needed for mesocosm operation (see Fig 3.1). Mesocosms frames were deployed by RV Alkor (KOSMOS2013) and RV Hespérides (KOSMOS2014), respectively. Please see the overview about KOSMOS2013 and KOSMOS2014 Expeditions detailed in (Bach et al. 2016b) and (Taucher et al. 2017a), respectively, whilst a standard mesocosms set-up is presented in Fig 3.1B.

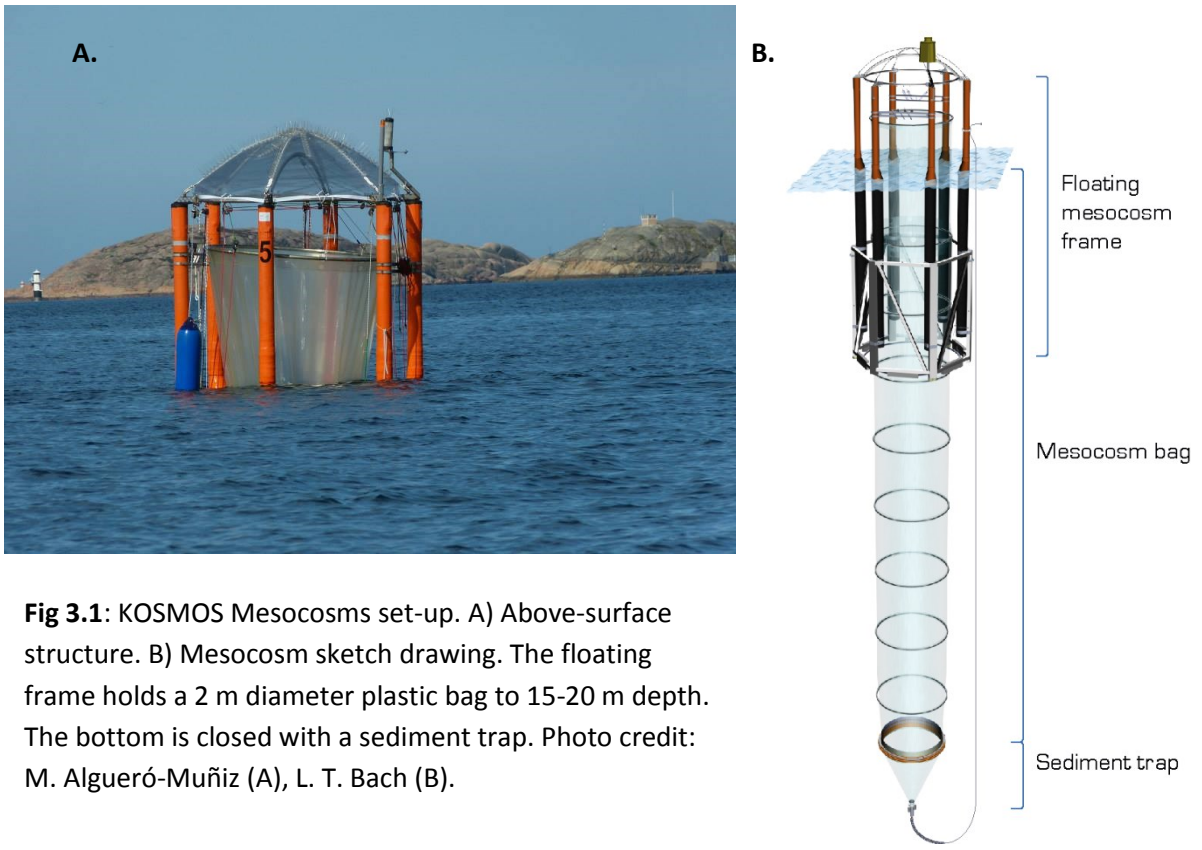

Fig 3.1: KOSMOS Mesocosms set-up. A) Above-surface structure. B) Mesocosm sketch drawing. The floating frame holds a 2 m diameter plastic bag to 15-20 m depth. The bottom is closed with a sediment trap. Photo credit: M. Algueró-Muñiz (A), L. T. Bach (B).

After deployment and mooring, both the upper and lower openings of the mesocoms bags were covered with meshes (3 mm mesh size) in order to exclude large zooplankton like fish larvae or jellyfish from the enclosed water body. Mesocosms were left floating in the water for ~4 days, then divers removed the meshes at the bottom and connected the lower part of the bags to the sediment traps. Afterwards, a ring same radius as the inside of the mesocosms structures was equipped with a 1mm mesh, and used as a cleaning device before the beginning of the experiments. Mesocosms maintenance was conducted on a weekly basis from the inside (using the same cleaning ring, without the mesh) and the outside (with scrubbers) in order to minimize growth of benthic organisms.

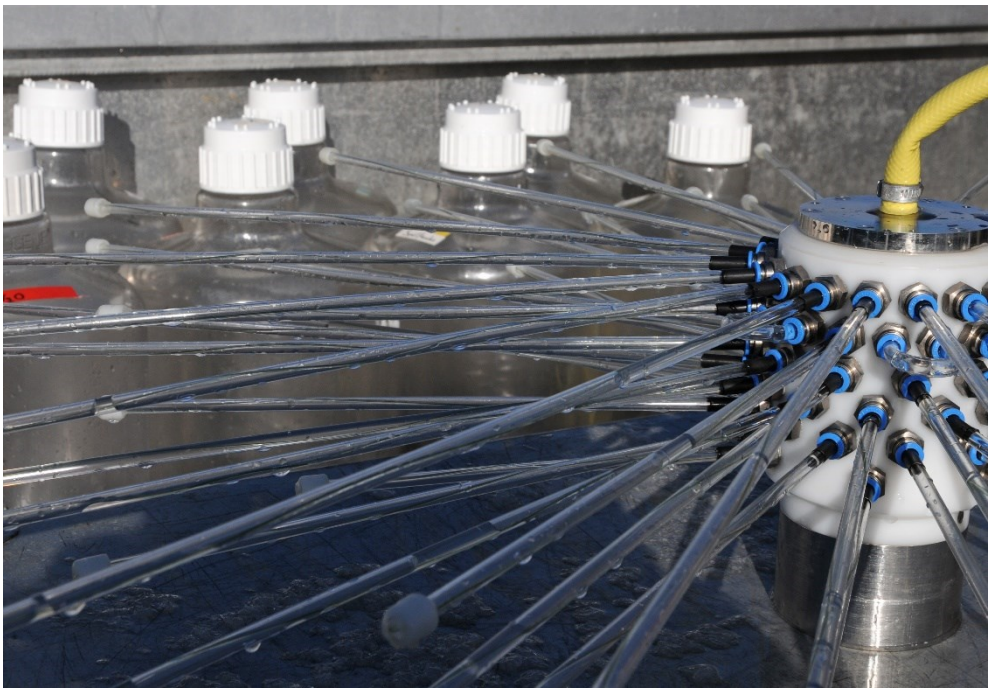

Fig 3.2: "The spider". Distribution device to pump the aerated water into the high CO_2 mesocoms. The multiple small tubes disperse the volume evenly within a radius of ~1 m. By pulling the spider up and down within each mesocosm, we ensured homogenous CO_2 enrichment throughout the entire water column. Photo credit: M. Nikolai.

In both experiments, target pCO_2 treatments were reached by adding CO_2-saturated sea water into the mesocosms (Riebesell et al. 2013). To do that, ~1500L filtered surface water were aerated with pCO_2 for at least 1 h to reach pH_{NBS} ~4 and subsequently transferred to 25 L gas-tight bottles for transportation to the mesocosms by boat. Then the aerated water was

pumped through a distribution device that we called “the spider” because of its shape, consisting in a central structure connected to multiple 1m long tubes (see Fig 3.2). By pulling the spider up and down, we ensured a homogenous pCO_2 enrichment thorough the entire water column of the mesocosms. After the initial CO_2 manipulation, further CO_2 additions were conducted during both experiments to account for loss of CO_2 through air-sea exchange. Mesocosms volume was estimated before the beginning of the experiment (t0) using salinity as a tracer, by adding precise amounts of saturated NaCl brine, as described by Czerny et al. (2013).

The experimental design during Gullmar Fjord KOSMOS2013 Expedition consisted on 10 mesocosms: 5 ambient mesocosms and 5 mesocosms under end-of-century pCO_2 levels (target = 1000 µatm) (Bach et al. 2016b). First CO_2 manipulation was carried out between t-1 and t4, and following CO_2 additions were made on a regular basis in the course of the experiment (day 17, 46, 48, 68 and 88) to compensate CO_2 loss, reaching an average of ~760 µatm during the 113 days that the experiment lasted.

During Gran Canaria KOSMOS2014 Expedition we created a pCO_2 gradient from current levels to end-of-century scenarios, representing IPCC predictions for medium (RCP 6.0) and high (RCP 8.5) pCO_2 levels (IPCC, 2013) with average values of ca. 390, 649 and 956 µatm, respectively (Taucher et al. 2017a). First CO_2 manipulation was carried out in four steps between t0 and t6, and subsequent additions were made during the course of the experiment (days 2, 4, 6, 21 and 38). The mean pCO_2 values per mesocosms between t1 and t55 were M1=369, M2=887, M3=563, M4=716, M5=448, M7=668, M8=1025 and M9=352 µatm, respectively. Analysing the pre-bloom phase of the experiment, we observed three pCO_2 groups occurring among the mesocosms so we run a K-means cluster analysis and the outcome showed three distinguishable clusters: low-pCO_2 (M1, M9, M5; K=460 µatm) medium-pCO_2 (M3, M7, M4; K=721 µatm) and high-pCO_2 levels (M2, M8; K=1111 µatm) (Fig 1A) which were used for the analyses presented throughout this paper. Unfortunately, the third high-pCO_2 mesocosm (M6=976 µatm) was lost on t27 due to a storm, so data are only available until that date.

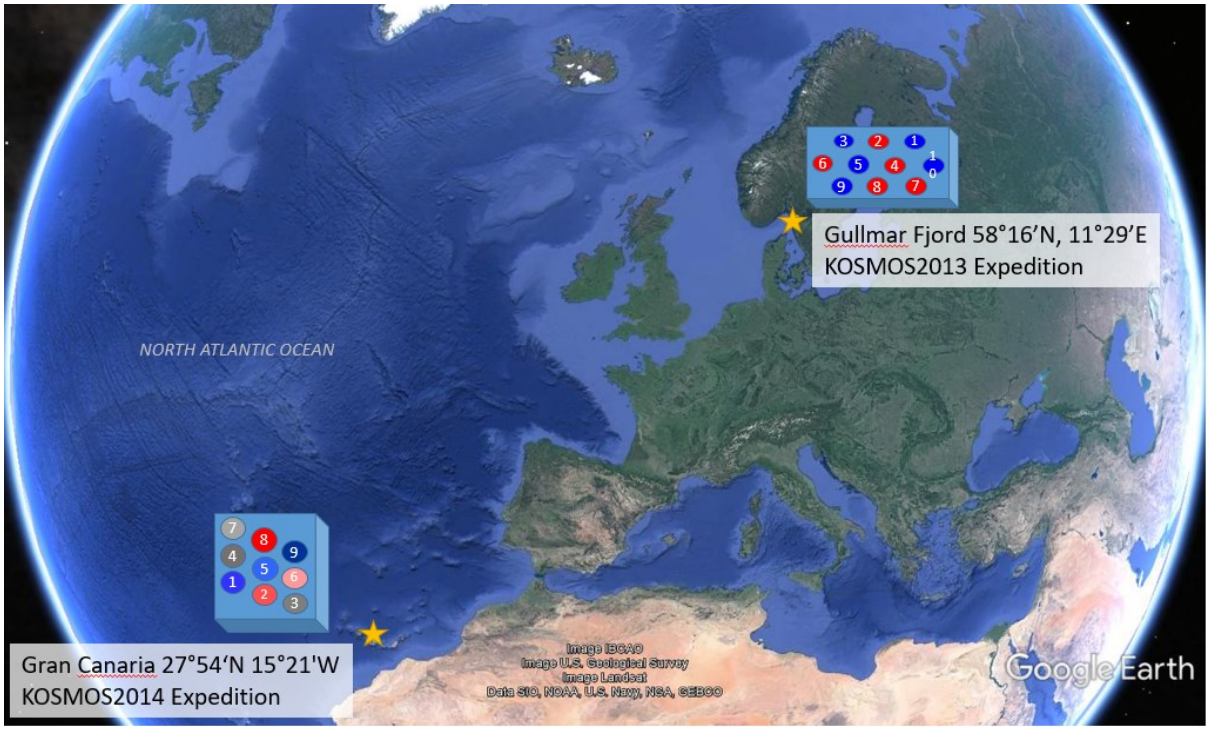

Fig 3.3: Study sites, both in North Atlantic Ocean: Gullmar Fjord KOSMOS2013 Expedition (North Sea) and Gran Canaria KOSMOS2014 Expedition (Subtropical North-east Atlantic. Yellow stars: mesocosms deployment sites. Source: Google Earth.

4. CHAPTERS

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

The chapters of this thesis are already published (Chapter 1, 3 and 4) or about to be submitted (Chapter 2) to scientific journals. This list serves as a clarification of my personal contribution to each publication:

- CHAPTER I

Ocean acidification effects on mesozooplankton community development: results from a long-term mesocosm experiment

Authors: Algueró-Muñiz M, Alvarez Fernandez S, Thor P, Bach LT, Esposito M, Horn HG, Ecker U, Langer JAF, Taucher J, Malzahn AM, Riebesell U, Boersma M.

Published in PLOS One, 2017;12(5):e0175851. doi: 10.1371/journal.pone.0175851

Contribution: Mesocosm experiments are inherently multi-person efforts. Hence, there was a large team responsible, for designing, planning, executing and analysing. MAM was responsible for the mesozooplankton part of the experiment, including field and laboratory work. Countings were done by MAM and UE. Biochemical analyses of the mesozooplankton samples were done by MAM and PT. Data analysis was conducted by SAF and MAM. MAM wrote the manuscript in close cooperation with all the co-authors.

- CHAPTER II

Impacts of ocean acidification on the development of a subtropical zooplankton community during oligotrophic and simulated bloom conditions

Authors: Algueró-Muñiz M, Horn HG, Alvarez-Fernadez S, Spisla C, Aberle-Malzahn N, Bach LT, Guan W, Achterberg E, Boersma M.

To be submitted to Frontiers in Marine Science.

Contribution: There was a large team responsible, for designing, planning, executing and analysing this mesocosms study. MAM was responsible for the mesozooplankton part of the experiment, including field work and onshore laboratory experiments. Microzooplankton analyses: HGH. Mesozooplankton analyses: MAM and CS. MAM compilated and arranged the data for the data analysis, which was conducted by SAF and MAM. MAM wrote the manuscript in close cooperation with all the co-authors.

- CHAPTER III

Direct and indirect effects of near-future pCO_2 levels on zooplankton dynamics.

Authors: Meunier CL, Algueró-Muñiz M, Horn HG, Lange JAF, Boersma M.

Published in Marine & Freshwater Research, 2016. doi: 10.1071/MF15296.

Contribution: MAM took part in performing the experiments, analysing the data and writing the manuscript.

- CHAPTER IV

Withstanding multiple stressors: ephyrae of the moon jellyfish (*Aurelia aurita*, Scyphozoa) in a high-temperature, high-CO_2 and low-oxygen environment.

Authors: Algueró-Muñiz M, Meunier CL, Holst S, Alvarez-Fernandez S, Boersma M.

Published in Marine Biology, 2016;163(9):1-12. doi: 10.1007/s00227-016-2958-z.

Contribution: MAM conceived and designed the experiment. MAM cultured the polyps until strobilation, and ran the experiment with CLM. Biochemical analyses and alkalinity measurements were done by MAM. MAM took part with SAF in analysing the data. MAM wrote the manuscript in close cooperation with all the co-authors.

Contribution of the PhD candidate in percentage of the total work load (100% for each of the following categories):

CHAPTER I

Experimental concept and design:	ca. 60%
Acquisition of (experimental) data:	ca. 70%
Data analysis and interpretation:	ca. 70%
Preparation of Figures and Tables:	ca. 80%
Drafting of the manuscript:	ca. 95%

CHAPTER II

Experimental concept and design:	ca. 80%
Acquisition of (experimental) data:	ca. 60%
Data analysis and interpretation:	ca. 70%
Preparation of Figures and Tables:	ca. 80%
Drafting of the manuscript:	ca. 95%

CHAPTER III

Experimental concept and design:	ca. 10%
Acquisition of (experimental) data:	ca. 25%
Data analysis and interpretation:	ca. 25%
Preparation of Figures and Tables:	ca. 0%
Drafting of the manuscript:	ca. 0%

CHAPTER IV

Experimental concept and design:	ca. 90%
Acquisition of (experimental) data:	ca. 90%
Data analysis and interpretation:	ca. 70%
Preparation of Figures and Tables:	ca. 70%
Drafting of the manuscript:	ca. 90%

CHAPTER I

Ocean acidification effects on mesozooplankton community development: results from a long-term mesocosm experiment

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Abstract

Ocean acidification may affect zooplankton directly by decreasing in pH, as well as indirectly via trophic pathways, where changes in carbon availability or pH effects on primary producers may cascade up the food web thereby altering ecosystem functioning and community composition. Here, we present results from a mesocosm experiment carried out during 113 days in the Gullmar Fjord, Skagerrak coast of Sweden, studying plankton responses to predicted end-of-century pCO_2 levels. We did not observe any pCO_2 effect on the diversity of the mesozooplankton community, but a positive pCO_2 effect on the total mesozooplankton abundance. Furthermore, we observed species-specific sensitivities to pCO_2 in the two major groups in this experiment, copepods and hydromedusae. Also stage-specific pCO_2 sensitivities were detected in copepods, with copepodites being the most responsive stage. Focusing on the most abundant species, *Pseudocalanus acuspes,* we observed that copepodites were significantly more abundant in the high-pCO_2 treatment during most of the experiment, probably fuelled by phytoplankton community responses to high-pCO_2 conditions. Physiological and reproductive output was analysed on *P. acuspes* females through two additional laboratory experiments, showing no pCO_2 effect on females' condition nor on egg hatching. Overall, our results suggest that the Gullmar Fjord mesozooplankton community structure is not expected to change much under a realistic end-of-century OA scenarios as used here. However, the positive pCO_2 effect detected on mesozooplankton abundance could potentially affect biomass transfer to higher trophic levels in the future.

1. Introduction

Continuous burning of fossils fuels is causing an increase of atmospheric carbon dioxide (CO_2), and current atmospheric pCO_2 values (ca. 400 µatm) are projected to reach levels of up to 1000 µatm in less than 100 years (IPCC 2013). Approximately one-third of the anthropogenic CO_2 has been taken up by the oceans (Sabine et al. 2004) leading to a reduction in pH (hence the term "ocean acidification" (Wolf-Gladrow et al. 1999; Caldeira and Wickett 2003)) and shifts in seawater carbonate chemistry (Doney et al. 2009). Coastal marine ecosystems may be less sensitive to increased CO_2 than open ocean regions, as the natural CO_2 fluctuation in these areas is already substantial (Hoegh-Guldberg and Bruno 2010; IPCC 2013). However, ocean acidification (OA) can interact with other natural and anthropogenic environmental processes such as warming (Hoegh-Guldberg et al. 2007), eutrophication (Wallace et al. 2014), and deoxygenation (Gobler and Baumann 2016), making it a potential threat in conjunction with other stressors. Furthermore, OA may affect zooplankton not only directly by decreases in pH, but also indirectly via trophic pathways (Boersma et al. 2008; Rossoll et al. 2012; Cripps et al. 2016). Consequently, both direct pH as well as pCO_2 effects on primary production (Dutkiewicz et al. 2015) may travel up the food web (Rossoll et al. 2012) therefore altering ecosystem functioning and community composition (e.g. (Lischka et al. 2011)).

Elevated pCO_2 in seawater may have positive effects on primary production, but at the same time impact marine organisms both via changes in calcification rates (Riebesell et al. 2000; Orr et al. 2005), and via disturbance to acid–base (metabolic) physiology (Fabry et al. 2008). Calcified secretions in marine fauna and flora are not only limited to skeletal $CaCO_3$ (thus, calcifiers *sensu stricto*) but there are other calcium-based structures that might be a target for low pH effects, such as, for example, the equilibrium organs (statoliths) in gelatinous zooplankton (Fabry et al. 2008). These organs are calcium magnesium phosphate crystals which may be affected by lowering pH (Purcell et al. 2007), as reported for statoliths of scyphomedusae (Winans and Purcell 2010).

Copepods are the most abundant marine planktonic metazoans and, together with microzooplankton, are the major primary consumers in most marine food webs, sustaining secondary consumers such as fish and jellyfish (Turner 2004; Landry and Calbet 2004). Copepods typically prefer larger and moving prey, i.e. they feed primarily on ciliates and

dinoflagellates than on diatoms (Calbet and Saiz 2005; Löder et al. 2011), with preferred sizes between 20 and 200 µm ((Kleppel 1993) and the references therein). As a result, they often switch from phytoplankton to microzooplankton over the course of a phytoplankton bloom (Löder et al. 2011) as larger prey items typically only become available later in the phytoplankton bloom, and even predate their offspring when resources are scarce (Boersma et al. 2014).

Previously, copepods were considered to be relatively tolerant to OA (Kurihara and Ishimatsu 2008; McConville et al. 2013), but several processes in copepods may in fact be affected by low pH, including metabolism (Pedersen et al. 2014b), pH balance (Meunier et al. 2016), reproduction (Cripps et al. 2014a), development (Pedersen et al. 2013), growth (Pedersen et al. 2014a) and survival (Lewis et al. 2013). Furthermore, diverse sensitivities to OA exist between different species and even between life stages within species (Isari et al. 2015a). Early life stages are most sensitive, resulting in a potential negative effect on survival and/or development (e.g. (Mayor et al. 2007; Cripps et al. 2014a; Meunier et al. 2016)). Different sensitivities to OA might also be related to copepod habitats, thus those copepod species more exposed to natural pH fluctuations (as vertical migrators or coastal species) might be more tolerant to OA than others (Lewis et al. 2013; Almén et al. 2014).

During the last decade, numerous studies dealing with the potential effects of high CO_2 on single species were published (e. g. (Mayor et al. 2007; Dorey et al. 2013)), while ecosystem-level impacts have attracted less attention. In order to assess future OA effects on natural communities, studies focused on ecological interactions (e.g. (Pedersen and Hansen 2003b; Rossoll et al. 2013; Lischka et al. 2015; Sala et al. 2015)), as well as long-term multigenerational experiments (Dupont et al. 2012; Scheinin et al. 2015; Thor and Dupont 2015) are of paramount importance. To investigate the effects of end-of-century pCO_2 levels on coastal pelagic ecosystems, we conducted a long-term mesocosm experiment in a boreal fjord. The present paper is part of the BIOACID II long-term mesocosm study PLoS Collection (Bach et al. 2016b). Here we focus on the natural mesozooplankton community, in particular on copepods and hydromedusae as the most abundant taxa. Testing the null hypothesis of no-effect, we assessed (1) mesozooplankton community development along the winter-to-summer plankton succession and the OA effects on the community interactions as well as (2)

temporal trends and high-CO_2 effects on species abundances, supported by two onshore experiments in the case of the most abundant copepod species, *Pseudocalanus* a*cuspes*.

2. Materials & Methods

2.1 Mesocosms setup and experimental design

Within the framework of the BIOACID II project (Biological Impacts of Ocean ACIDification), this study was part of the ”BIOACID II long-term mesocosm study”, which was conducted from January to July 2013 in the Gullmar Fjord (58°15’ N, 11°28’ E), on the Swedish Skagerrak coast (Bach et al. 2016b). We deployed ten mesocosms (KOSMOS, M1-M10: “Kiel Off-Shore Mesocosms for future Ocean Simulation”, (Riebesell et al. 2013; Sswat et al. 2015)) in the fjord to study the effect of changing carbonate chemistry conditions on mesozooplankton community development. The experimental units consisted of large enclosed water volumes (~50 m^3), five of them used as controls (ambient pCO_2 levels = ca. 380 µatm), and the other five were CO_2-enriched in levels adjusted to realistic end-of-century scenarios (RCP 6.0 (IPCC 2013)). Mesocosms were sealed by sediment traps, installed at the bottom of each mesocosm bag. Target pCO_2 was reached at the beginning of the experiment by adding CO_2 saturated seawater to the mesocosms. Subsequent additions were made on a regular basis in the course of the experiment (day 17, 46, 48, 68 and 88) to compensate for CO_2 loss through outgassing. We established realistic end-of-century pCO_2 levels (average = ca. 760 µatm) over the study period (see Fig I-1a, (Bach et al. 2016b)). Regular sampling every 2nd day included CTD casts, water column sampling, and sediment sampling. Water column samples were collected with integrating water samplers (IWS, Hydrobios), which collect a total volume of 5 L from 0-17 m depth evenly through the water column. This water was used for nutrient analyses, pigment analysis, and microzooplankton microscopy. All analyses are described in detail in (Bach et al. 2016b) within this PLoS Collection. Briefly, nutrient (NO_3^-+ NO_2^-) concentrations (Fig I-1b, (Bach et al. 2016b)) were measured with a SEAL Analytical QuAAtro AutoAnalyzer and a SEAL Analytical XY2 autosampler. Pigment extracts were used for analysis by means of reverse phase high performance liquid chromatography (HPLC) (Fig I-1c, (Bach et al. 2016b)). Every eight days, microzooplankton samples were taken from the IWS carboys, immediately fixed with acidic Lugol’s solution and stored dark until identification (Fig I-1d, (Horn et al. 2016b)).

Results presented here correspond to t_1 (10th March) up to t_{103} (20th June) of the 113 days that the mesocosms experiment lasted (Bach et al. 2016b).

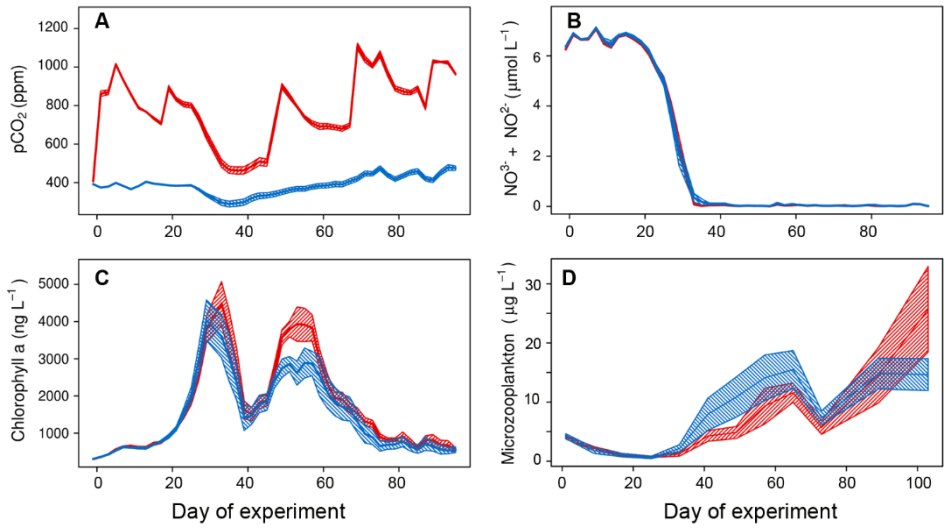

Fig I-1: Abiotic and biotic factors potentially affecting mesozooplankton community along the experiment. A) *in situ* pCO_2 levels, B) nutrients (NO_3^-+ NO_2^-), C) chlorophyll *a,* and D) microzooplankton abundances (ciliates and heterotrophic dinoflagellates). Colour code: red = treatment (~760 µatm pCO_2), blue = control (ambient conditions). Solid lines = mean values; striped area = standard error of the mean.

2.2 Mesozooplankton sampling

The mesozooplankton community was sampled in the mesocosms and the fjord by vertical net hauls with an Apstein net (55µm mesh size, 17 cm diameter) equipped with a closed cod end, sampling a total volume of 385 L. Sampling depth was restricted to the upper 17m to avoid resuspension of the material accumulated in the sediment traps, at 20m depth. One net haul per mesocosm was taken once every eight days, within a narrow time-window (1 to 3 p.m.) to avoid differences in the community composition caused by diel vertical migration. Note that sampling frequency was lower than for other water column samples to avoid overharvesting of the plankton community. Samples were rinsed on board with filtered sea-water, collected in containers and brought to the laboratory, where samples were preserved in 4% formaldehyde buffered with sodium tetraborate. For transportation during summer time, the samples were placed in cooling boxes until fixation of the organisms.

During analysis, organisms were sorted using a stereomicroscope (Olympus SZX16) and classified to the lowest possible taxonomical level, including gender in the case of adult copepods. Copepodites and adults were classified to species level whereas nauplii from different species were pooled together. Taxonomical analyses were carried out focusing on copepods (Sars 1901-1903; Sars 1903-1911; Sars 1913-1918; Razouls et al. 2005) and hydromedusae (Bouillon et al. 2006; Schuchert 2007; Schuchert 2010) as the most abundant groups. Every sample was sieved through 50 µm mesh, rinsed with tap water and poured into a calibrated beaker, where organisms were well mixed before taking a 5% aliquot with a Hensen Stempel pipette (2000). Counting was restricted to 5% (one aliquote) or 10% (two aliquots) of the total sample for the most abundant groups (nauplii, *P. acuspes* adults and *P. acuspes* copepodites) when more than 200 individuals were counted in the first aliquot. Otherwise the subsampling procedure was repeated, counting a maximum of a 15% of the total sample for all species.

Since some organisms characteristic to a winter-to-summer succession might not have been included when the experiment started, the community within the mesocosms was enriched by the addition of 22 L of fjord water every fourth day (Bach et al. 2016b). Likewise Atlantic herring (*Clupea harengus*) eggs and green sea urchin (*Strongylocentrotus droebachiensis*) gastrulae were artificially added to each mesocosms on t_{48} and t_{56} respectively (Bach et al. 2016b) according to the time of the year that these groups would have been part of the natural fjord community. Densities of herring eggs introduced in the mesocosms were ~70 - 108 eggs per m^3 and peak egg-hatching was estimated to occur around t_{63}, with a final number of 1608 ± 237 hatched larvae per mesocosms, i. e. ~27 - 37 larvae per m^3 (Sswat et al.). These larval densities are within the natural range for the North Sea (Alvarez-Fernandez et al. 2015). Sea urchin gastrulae were obtained in the onshore laboratory, introduced in the mesocosms (~110 sea urchin gastrulae per m^3) and subsequently monitored from the mesozooplankton net tows on a weekly basis. An in depth analyses of Atlantic herring and green sea urchin larvae development are provided by Sswat et al. (Sswat et al.) within the framework of this PLoS Collection and Dupont et al. (unpubl. data).

2.3 *P. acuspes* condition experiments

Copepods were the most abundant group within the mesozooplankton community during the whole experiment, and the calanoid copepod *P. acuspes* was the most abundant species. To gain insights in *P. acuspes*' physiological response to simulated OA we conducted two additional incubation experiments during the pre-bloom (March, t_{19}) and senescence phase (May, t_{59}) of the phytoplankton community (Fig I-1). Every mesocosms was sampled by an extra net haul (see 2.2), and *P. acuspes* females were sorted immediately and subsequently incubated in a cold room adjusted to the average *in situ* temperature (t_{19}: 3°C and t_{59}: 5 °C (Bach et al. 2016b)) for offspring viability monitoring (*n*=12) and respiration measurements (*n*=5), or preserved for carbon content analyses (*n*=20). Normally swimming females with undamaged eggs (60 females per treatment) were selected and initial clutch sizes were noted prior incubation to assess hatching rates. We aimed to incubate 12 females per mesocosms (i. e., 60 females per treatment), but this was not achieved in all cases due to the scarcity of egg carrying females within some samples or due to mortality of the females after 24h. Considering that incubation in small volumes does not affect egg production (Niehoff et al. 1999) , females were incubated for 48h in 6-well plates, one female per well, in starvation and simulated field temperature. No additional pCO_2 treatment was necessary because the aim of this side experiment was to analyse the memory effects of increased pCO_2 on females in the mesocosm rather than effects on the eggs themselves. Clutch size and survival of the females were recorded each day during the condition experiments. Prosome length of all incubated females was measured upon termination of the experiment.

Respiration rates of five egg-carrying females per mesocosm (i. e. 25 animals per treatment) were measured in the cold room. Females were transferred to 1.6 mL vials equipped with fluorescent O_2 foil discs (PSt3 spots, PreSens Precision Sensing, Germany) and filled with seawater adjusted to the pCO_2 levels from corresponding mesocosms, based on the immediately preceding carbonate chemistry measurements in the mesocosms (Bach et al. 2016b). Vials were then sealed with Teflon caps and O_2 concentrations were measured at 0, 3, and 6 hours using a Fibox 3 optode system. Respiration rates were calculated by subtracting the average oxygen depletion rate measured in five controls from the oxygen depletion rate in the vials holding copepods, multiplying by vial volume and dividing by number of individuals in each vial. Prior testing of the optode system at 5 °C showed a 2 min 95% reaction time, i.e.

the period of time taken before the output reached within 5% of the final oxygen concentration value (as estimated by exponential regression). Therefore, at every sampling, oxygen concentrations were read for three minutes, and an average of values read during the last minute was used for calculations.

To analyse carbon content, 20 non-ovigerous *P. acuspes* females were sorted from each mesocosm sample (i. e. 100 animals per treatment). The females were briefly rinsed in Milli-Q water to remove the excess of salt, and preserved in pre-weighted tin cups, which were in time dried (60°C) and preserved in an desiccator until analysed. Weights were obtained with a microbalance (Sartorius SC2). A Vario MICRO cube CHN analyser (Elementar) was used to measure carbon content.

2.4 Statistical analysis

To study Gullmar Fjord's mesozooplankton community we firstly calculated species diversity for every mesocosm, which were compared using general linear models (GLMs) to detect any differences among treatments (high-pCO_2, ambient). Subsequently, we analysed total abundances and abundances from the most frequent mesozooplankton species using general additive mixed models (GAMMs) to analyse the effect of the treatments as well as temporal trends. We compared the development of the community between treatments by a non-metric multidimensional analysis (NMDS) followed by a similarity analysis (ANOSIM). Finally, focusing on the most abundant species in the mesocosms (*P. acuspes*), we compared productivity and females' condition between treatments by using GLMs.

Mesozooplankton diversity in mesocosms was calculated by using the Simpson's Diversity Index (*D*) for finite communities. This index ranges from 0 to 1, and it is adapted to the form 1-*D* for a more intuitive interpretation of the results, thus higher values indicate higher sample diversity. Males, females and copepodites of the same copepod species were pooled together. Nauplii were assumed to be *P. acuspes* since this species accounted for > 90% of the copepod abundance during the whole experiment. General linear models (GLMs) were fitted to the Simpson's indices to determine the dependence of diversity 1-*D* on time and

pCO_2. Calculations of *D* were performed in the vegan package of the R environment (Oksanen et al. 2012).

A multivariate analysis (NMDS) was used to describe the changes in the mesozooplankton community throughout the mesocosm experiment. NMDS is an ordination technique which represents, in an *n*-dimensional space, the dissimilarities obtained from an abundance data matrix (Zuur et al. 2009). NMDS takes a rank based approach, being more robust to datasets like the one used here, but as a consequence all the information about the magnitude of distances is lost. NMDS is therefore useful to represent the dissimilarities, and assess the influence of the treatment in the evolution of the community. However, due to the lack of magnitude, this technique is not ideal to evaluate the influence of environmental gradients on community changes (Legendre and Anderson 1999). The treatment effect was assessed by using permutation tests on the community position in the NMDS space, by checking if the area of clusters formed by the treatment in the NMDS were smaller than randomized samples of the same size (Legendre and Anderson 1999). In a complementary approach, we applied an ANalysis Of SIMilarity (ANOSIM) test (Clarke 1993) as a post-analysis to compare the mean of ranked dissimilarities between treatments (high-pCO_2, ambient) to the mean of ranked dissimilarities within treatments. This analysis tests the assumption of ranges of (ranked) dissimilarities within groups are equal, or at least very similar (Buttigieg and Ramette 2014).

Only those species that were present in at least one of the mesocosms for more than nine sampling days (2/3 of the number of days sampled) were used for temporal trends and multivariate analyses. By this criterion, the species selected for the analyses were: the hydromedusae *Aglantha digitale* and *Hybocodon prolifer,* and the females, males and copepodites of the copepod species *Oithona similis, Temora longicornis,* and *P. acuspes.* The aggregated copepod nauplii (pooled in one group and not identified to species level) were also included in these analyses.

To describe the temporal trends of each species during the mesocosm experiment we used GAMMs (Wood 2006; Zuur et al. 2009) with a Poisson distribution and with a logarithmic transformation. Four different kinds of models were fitted to each abundance group (Table I-1). Each of these models allowed the temporal trends to vary differently between treatments, representing (a) no difference between treatments ($\alpha + f$), (b) differences in

temporal trends but not in abundance ($\alpha + f_T$) (c) difference in absolute abundance but not in temporal trends ($\alpha_T + f$) and (d) difference both in absolute abundance and temporal trends ($\alpha_T + f_T$). In this way potential differences between pCO_2 and ambient mesocosms could be detected as either increase/decrease of overall abundance or changes in phenology. All models were fitted with an autocorrelation structure of first order to account for temporal autocorrelation in the data, and the specific mesocosm was used as a random intercept as the focus of the analyses was not the differences between mesocosms, but between treatments (Zuur et al. 2009). The models were compared by means of the Akaike Information Criterion (AIC). AIC takes into account both the goodness of fit of the model and model complexity, with lower AIC values indicating models with a better ratio between the explained variance and the number of variables (Wood 2006). For each species, the model with the lowest AIC was considered to better represent the temporal trends during the experiment, while avoiding overfitting the data.

Table I-1: Generalized additive mixed model (GAMM) structures

$\boldsymbol{\alpha + f}$	Temporal trend and absolute abundances are treatment-independent *(*Model *Trtmt_indep)*
$\boldsymbol{\alpha + f_T}$	Temporal trends depend on the treatment, but absolute abundances are treatment independent *(*Model *Trtmt_trend)*
$\boldsymbol{\alpha_T + f}$	Absolute abundances depend on the treatment, temporal trends are treatment independent *(*Model *Trtmt_absAb)*
$\boldsymbol{\alpha_T + f_T}$	Both absolute abundances and temporal trends are affected by the treatment *(*Model *Trtmt_absAb_trend)*

In the case of copepods, we analysed the effects of the end-of-century pCO_2 treatment on *P. acuspes* productivity by estimating a *nauplii-to-adult* ratio. Afterwards, GLMs were fitted to these ratios. The differences in the physiological and reproductive condition of *P. acuspes* females were analysed by GLMs comparing the potential effect of treatment and month in respiration rates, carbon content, prosome length, clutch size and hatching success. The effect of the time of the year (March and May), treatment and their interaction was considered in the models.

We used R (version 3.0.2, (Team 2012)) to fit abundances data with the GAMMs and GLMs. The significance level for all statistical analysis was set to $p < 0.05$.

3. Results

3.1 Mesozooplankton community: composition, diversity and development

The mesozooplankton community comprised 27 different species and taxonomic groups (for a complete taxon list, see Table I-2). The morphological classification of the most abundant groups (copepods and hydromedusae) was consistent with the genetic analyses conducted during the experiment (see Langer et al. (2017) for more details). Copepods were the most abundant group throughout the experiment, representing 93 - 97% of the total abundances. *P. acuspes* was the dominant species in terms of abundance; based on the sum of adults and copepodites, *P. acuspes* represented 99.9% of the total copepod population at the beginning of the experiment and 33.6% at the end. Together with *P. acuspes*, only two other copepod species (*T. longicornis, O. similis*) and two hydromedusae (*A. digitale, H. prolifer*) were regularly recorded in our quantitative analyses. Other copepods and hydromedusae, polychaetae, chaetognatha, and appendicularians, as well as echinodermata, pteropoda, fish (larvae, eggs), bivalvia, cirripedia, and cladocera were rare (counted in less than 2/3 of the number of days sampled) or very rare (recorded in less than 3 sampling days during the experiment) in the studied community.

Mesozooplankton abundances (Fig I-2A) increased after the first phytoplankton built-up (t_{17}), and decreased during the phytoplankton post-bloom phase (t_{41}-t_{77}) and before microzooplankton increase (t_{81}) (Fig I-1C, D). GAMM analysis showed a treatment effect in total mesozooplankton abundances, which were higher under acidification scenarios (*Trtmt_abdAb*, Table I-3). Averaged total catch (M1-M10) at the beginning of the experiment (t_1) was 14571 ± 2857 individuals per m^3, reached maximum in t_{49} (136342 ± 24451 individuals per m^3), to decrease until minimum levels at t_{103} (9497 ± 3111 individuals per m^3). Mesozooplankton biodiversity (1-*D*) was low during the experiment (Fig I-2B), with average values of 0.094 ± 0.018 in ambient conditions and 0.098 ± 0.043 in the high-pCO_2 mesocosms. No differences between ambient conditions and high-pCO_2 treatment were observed (non-

significant effect of treatment in a GLM). Independently from the pCO_2 treatment, Simpson's index (1-*D*) stayed below 0.1 in both treatments until t_{81}. Then the index increased, with maxima on t_{103} (0.552 ± 0.045 in ambient and 0.535 ± 0.126 in high-pCO_2, respectively).

Table I-2: Complete list of species and taxa present in the mesocosms registered throughout the study period. Based on our records, species were classified as common (recorded on at least 9 sampling days, hence used for the GAMM analyses), rare (counted on 3 to 9 sampling days) or very rare (on less than 3 sampling days). C= common, R= rare, VR= very rare.

	Taxonomic groups	Records
1	*Aglantha digitale*	C
2	*Hybocodon prolifer*	C
3	*Sarsia tubulosa*	VR
4	*Rathkea octopunctata*	VR
5	*Obelia* sp.	VR
6	*Phialella quadrata*	VR
7	Bivalvia	VR
8	Pteropoda	R
9	Polychaeta	R
10	*Evadne* sp.	R
11	*Podon* sp.	R
12	Copepod nauplii	C
13	*Pseudocalanus acuspes*	C
14	*Temora longicornis*	C
15	*Oithona similis*	C
16	*Acartia clausi*	R
17	*Tisbe* sp.	R
18	*Centropages* cf. *hamatus*	R
19	*Calanus* sp.	VR
20	*Monstrilla* sp.	VR
21	*Ectinosoma* sp	R
22	*Parasagitta elegans*	R
23	Cirripedia	R
24	Ophiopluteus larvae	VR
25	Sea urchin larvae and juveniles	R
26	*Oikopleura dioica*	R
27	Teleostei (fish larvae)	VR

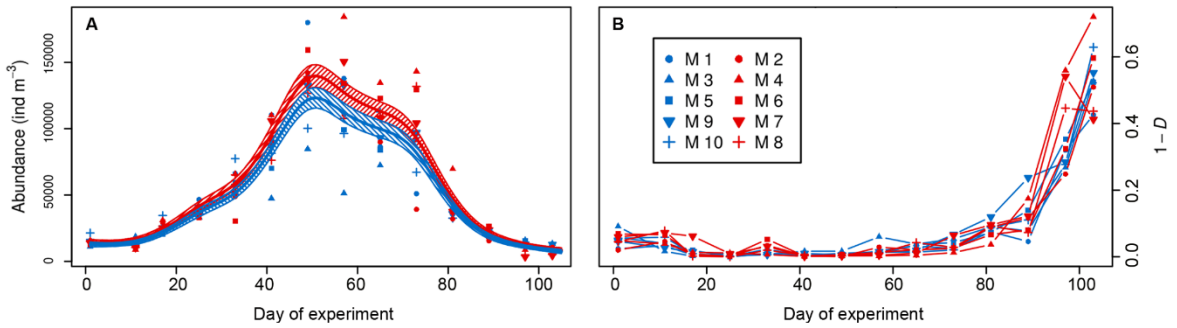

Fig I-2: Mesozooplankton community. A) Mesozooplankton abundances. Solid lines = prediction from Generalized Additive Mixed Models (GAMMs) (smoother trends *p*-value < 0.05) with ambient and high-pCO_2 mesocosms separately; striped area = confidence interval. B) Simpson's Diversity Index (1-*D*) in relation to pCO_2 levels within the mesocosms along the study period. Symbols and colours (blue = ambient; red = high-pCO_2 treatment) identify each mesocosm.

The 2-dimensional representation of the community did not show different patterns between treatments (Fig I-3). Permutation tests (with 999 permutations) did not show the areas (i. e. clusters of samples) representing the treatment to be significantly smaller than randomized areas, indicating no treatment effect in the ordination. On the contrary, areas representing the sampling day (Fig I-3) were significantly smaller than randomized areas using the same test. This result indicates clear community differences throughout the study period. Results from the ANOSIM test (*p*-value = 0.322) matched with the NMDS, suggesting that there was no significant difference between the community development under the high-pCO_2 treatment and the ambient conditions.

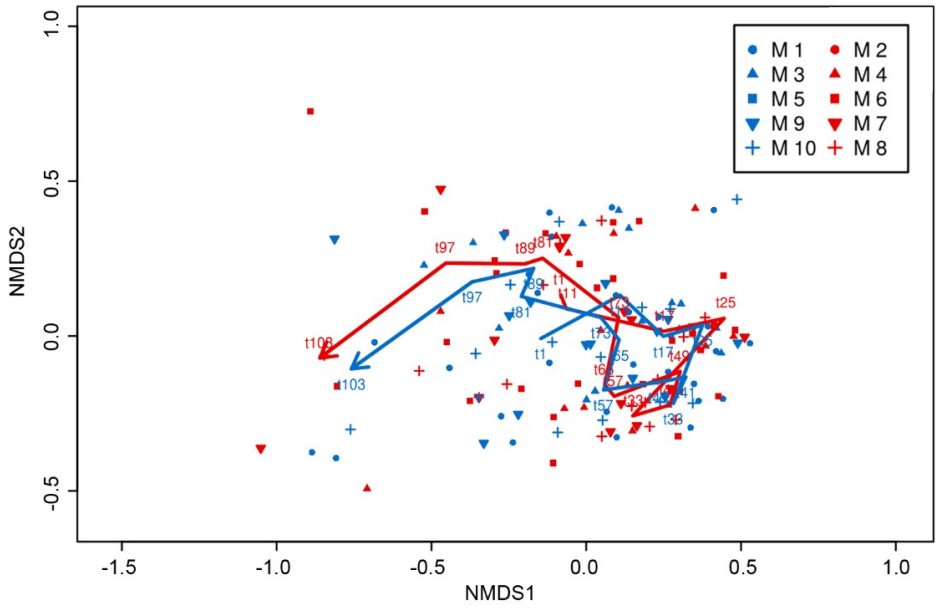

Fig I-3: Non-metric Multidimensional Scaling analysis (NMDS) of the mesozooplankton community (stress value = 0.17). Colour code: red = treatment (~760 µatm pCO_2), blue = control (ambient conditions). Sampling days represented as *t-day*; lines represent patterns. The underlying data implemented in the analysis are shown in Fig I-1.

3.2 Species abundances

Temporal trends of the selected species were analysed by using GAMMs (Figs I-4 and I-5; Table I-3). The model selection procedure discerned whether there was a difference in the temporal trends and abundances in between the two different treatments (i.e. high or ambient pCO_2).

There was no pCO_2 effect on the abundance of adult *P. acuspes* and *T. longicornis* but copepodite stages of both species responded to increased pCO_2. *P. acuspes* adults did not show differences in abundances nor in temporal trends between treatments (Table I-3 *Trtmt_indep* for both males and females; Fig I-4A, B). However, the absolute abundance of *P. acuspes* copepodites differed between treatments, being higher under the high-pCO_2 treatment (Table I-3 *Trtmt_absAb*; Fig I-4C). Abundance of *T. longicornis* adults did not show a difference between treatments (Fig I-4D, E); even though the selected model showed slightly higher abundances of *T. longicornis* females in the high-pCO_2 mesocosms (Table I-3 *Trtmt_absAb*; Fig I-4D), the confidence intervals of the modelled abundances were overlapping throughout the study period. This indicates that the difference were small, and probably caused by extreme values at the end of the experiment. Only *T. longicornis* copepodites (Table I-3 *Trtmt_absAb_trend*; Fig I-4F) showed different absolute abundances and a different temporal trend between treatments, being more abundant in the ambient pCO_2 mesocosms, particularly during the last 20 days of the study. *O. similis* adults negatively responded to the elevated pCO_2 conditions with an earlier abundance decrease towards the end of the experiment (Fig I-4G, H). In case of *O. similis* males the absolute abundance and the temporal trend were negatively affected by the high-pCO_2 treatment (Table I-3 *Trtmt_absAb_trend*). However, this effect was not detected on *O. similis* copepodites (Table I-3 *Trtmt_indep*; Fig I-4I), which showed no significant difference between both treatments. Copepod nauplii, the most abundant group in the mesozooplankton (Fig I-4J), did not show a difference in temporal trends nor abundance between treatments (Table I-3 *Trtmt_indep*).

Table I-3: Mesozooplankton community models selection. Generalized Additive Mixed Models (GAMMs) for the mesozooplankton community: a) $\alpha + f$, no difference between treatments (Model *Trtmt_indep*), b) $\alpha + f_T$, pCO_2 treatment effect on temporal trends but not in abundance (Model *Trtmt_trend*), c) $\alpha_T + f$, pCO_2 treatment effect on absolute abundance but not on temporal trends (Model *Trtmt_absAb*) and d) $\alpha_T + f_T$, treatment causes differences both in absolute abundance and seasonal trends (Model *Trtmt_absAb_trend*). Only those species that were present in at least one of the mesocosms more than 9 days (2/3 of the number of days sampled) and only convergent models were used for this analyses. The smoother of all selected models had a *p*-value < 0.05. For each species, the model with the lowest AIC (boldface) was considered to better represent the temporal trend during the experiment. Hyphens (-) indicate non-convergent models.

Taxa	Model type	R^2	AIC	Taxa	Model type	R^2	AIC
nauplii	***Trtmt_indep***	**0.855**	**257.797**	*T. longicornis* copepodites	*Trtmt_indep*	0.123	544.681
	Trtmt_trend	0.855	278.645		*Trtmt_trend*	0.127	540.113
	Trtmt_absAb	0.859	258.568		*Trtmt_absAb*	0.169	544.147
	Trtmt_absAb_trend	0.854	279.925		***Trtmt_absAb_trend***	**0.122**	**536.422**
P. acuspes ♀	***Trtmt_indep***	**0.441**	**189.89**	*O. similis* ♀	*Trtmt_indep*	0.558	463.501
	Trtmt_trend	0.491	195.135		***Trtmt_trend***	**0.583**	**445.861**
	Trtmt_absAb	0.443	191.887		*Trtmt_absAb*	0.552	465.903
	Trtmt_absAb_trend	0.5	197.739		*Trtmt_absAb_trend*	0.582	448.497
P. acuspes ♂	***Trtmt_indep***	**0.564**	**282.254**	*O. similis* ♂	*Trtmt_indep*	0.605	484.982
	Trtmt_trend	0.586	307.326		*Trtmt_trend*	0.635	482.307
	Trtmt_absAb	0.573	283.754		*Trtmt_absAb*	0.599	482.24
	Trtmt_absAb_trend	0.586	310.298		***Trtmt_absAb_trend***	**0.633**	**479.176**
P. acuspes copepodites	*Trtmt_indep*	0.727	210.277	*O. similis* copepodites	***Trtmt_indep***	**0.767**	**447.67**
	Trtmt_trend	0.752	232.495		*Trtmt_trend*	0.759	469.749
	Trtmt_absAb	**0.76**	**209.844**		*Trtmt_absAb*	0.766	449.509
	Trtmt_absAb_trend	0.75	234.226		*Trtmt_absAb_trend*	0.758	471.615
T. longicornis ♀	*Trtmt_indep*	-	-	*A. digitale*	*Trtmt_indep*	0.118	735.989
	Trtmt_trend	-	-		***Trtmt_trend***	**0.114**	**734.663**
	Trtmt_absAb	**0.044**	**635.237**		*Trtmt_absAb*	0.11	736.248
	Trtmt_absAb_trend	0.197	668.866		*Trtmt_absAb_trend*	0.11	739.801
T. longicornis ♂	***Trtmt_indep***	**0.157**	**614.175**	*H. prolifer*	*Trtmt_indep*	0.083	811.073
	Trtmt_trend	-	-		*Trtmt_trend*	0.151	764.543
	Trtmt_absAb	0.148	615.588		*Trtmt_absAb*	0.19	812.093
	Trtmt_absAb_trend	0.069	614.303		***Trtmt_absAb_trend***	**0.173**	**764.455**
Total catch	*Trtmt_indep*	0.852	92.57				
	Trtmt_trend	0.867	104.36				
	Trtmt_absAb	**0.868**	**91.95**				
	Trtmt_absAb_trend	0.866	106.35				

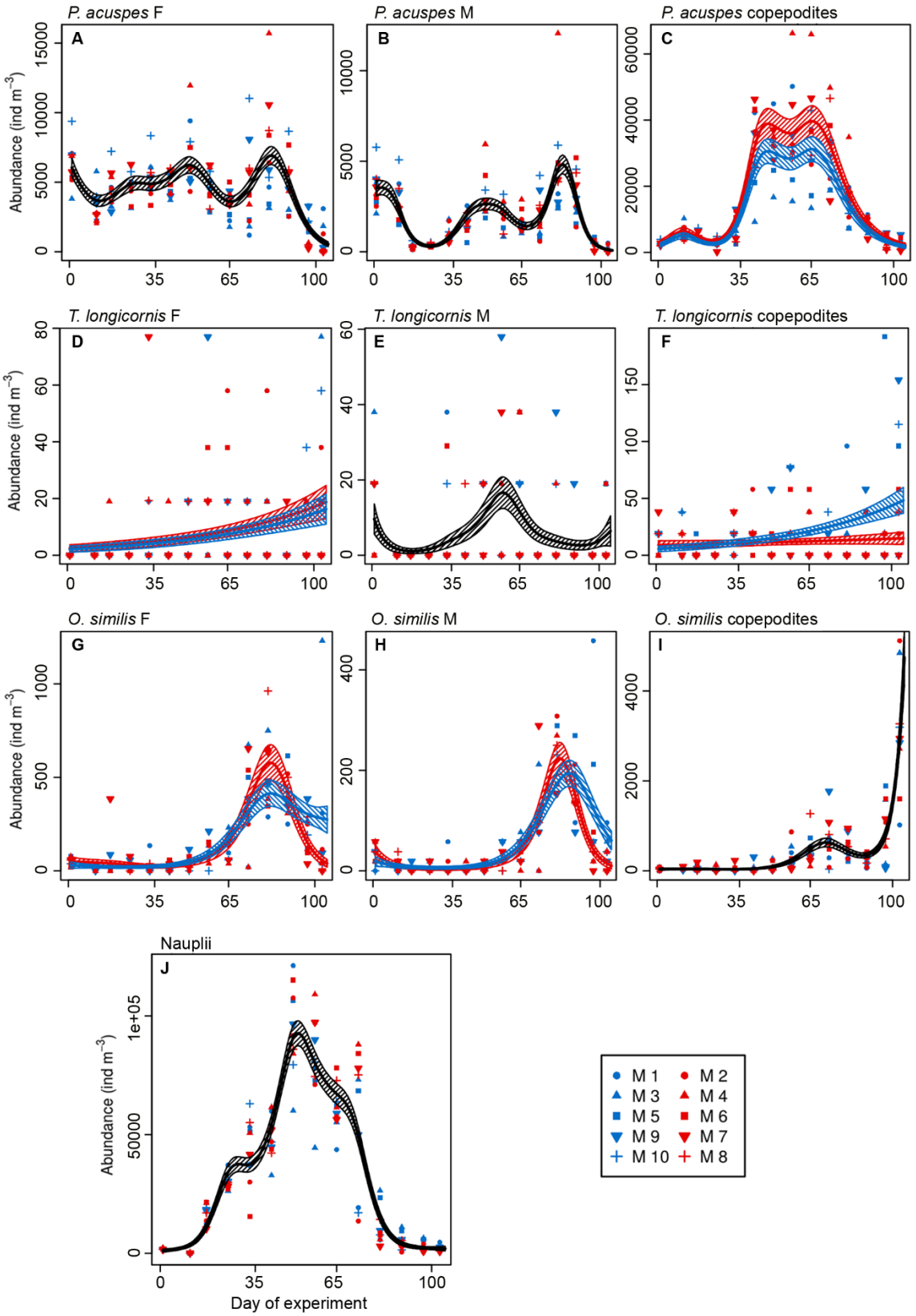

Fig I-4: Copepod abundances along the study period. A) *P. acuspes* females, B) *P. acuspes* males, C) *P. acuspes* copepodites, D) *T. longicornis* females, E) *T. longicornis* males, F) *T. longicornis* copepodites, G) *O. similis* females, H) *O. similis* males, I) *O. similis* copepodites, J) nauplii. Colour code: red = treatment (~760 µatm pCO_2), blue = control (ambient conditions). M = mesocosms. Solid lines =

prediction from Generalized Additive Mixed Models (GAMMs) (smoother trends *p*-value < 0.05) with the ambient and high-pCO_2 mesocosms shown separately; striped area = confidence interval. Black lines indicate that the prediction of the model for high-pCO_2 treatment and ambient conditions are the same.

When analysing abundances in certain time-points, we could detect different pCO_2 effects that were not detected by the GAMMs. In the case of *P. acuspes,* adult copepods were significantly more abundant on t_{81} (*t*-test, *p*-value = 0.010), but the effect disappeared afterwards. Different responses were also observed on nauplii abundances, which were significantly higher under high-pCO_2 conditions between t_{49} and t_{65} (*t*-test, *p*-value = 0.03), whilst we did not detect differences in abundances between treatments when analysing abundances from t_{65} until the end of the experiment (*t*-test, *p*-value = 0.622).

In the case of both hydromedusa species, we also detected species-specific pCO_2 effects (Fig I-5, Table I-3). Under the high-pCO_2 treatment, *H. prolifer* abundance was lower; the GAMM detected an effect not only on the temporal trend, but also on the abundances of this species (Table I-3 *Trtmt_absAb_trend*). The model representing *A. digitale* also showed a different temporal trend between treatments (Table I-3 *Trtmt_trend*) despite of the confidence intervals overlapping of both patterns.

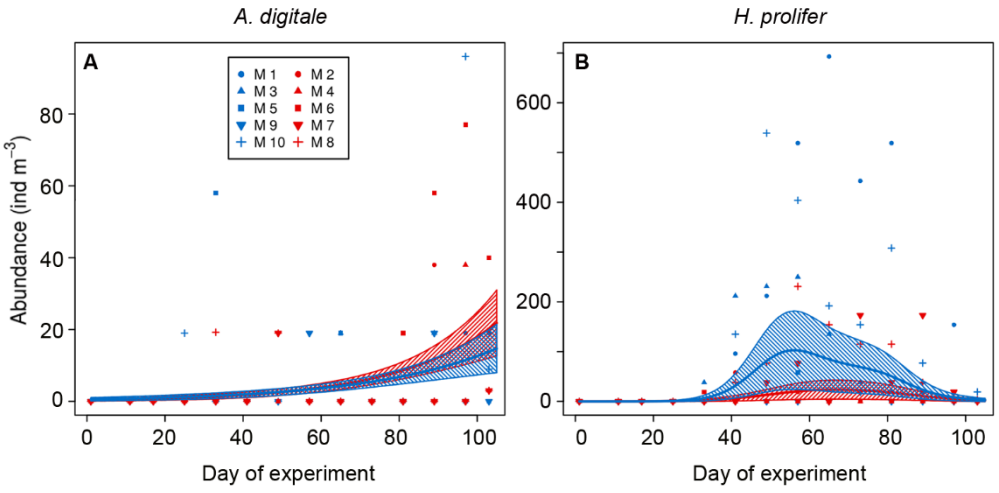

Fig I-5: Hydromedusae abundances along the study period. A) *A. digitale*, B) *H. prolifer.* Colour code: red = treatment (~760 µatm pCO_2), blue = control (ambient conditions). M = mesocosms. Solid lines = prediction from Generalized Additive Mixed Models (GAMMs) (smoother trends *p*-value < 0.05), with the ambient and high-pCO_2 mesocosms shown separately; striped area = confidence interval.

To sum up, after analysing the abundance of each species under high-pCO_2 conditions during the whole study period we observed positive (*P. acuspes* copepodites, *A. digitale*), negative (*T. longicornis* copepodites, *H. prolifer*, *O. similis* adults) and no effects of elevated pCO_2 (nauplii, *P. acuspes* and *T. longicornis* adults, *O. similis* copepodites). It is worth mentioning that the predictive power (R^2) of these models was low in some cases (see Table I-3) due to the complete absence of some species in some mesocosms. However, the models represented well the overall trend differences between treatments (Figs I-4 and I-5). Differences between treatments were at times significant for specific time periods.

3.3 *P. acuspes*: productivity and females' condition

Copepod productivity was assessed by computing the ratio between nauplii and adults for the most abundant species, *P. acuspes*. We calculated the *nauplii-to-adult* ratio from t_{17} until the end of the experiment, since the fraction < 200 µm was preserved only from t_{17} on. At a significance level of 0.05, no differences in this ratio between the ambient and high-pCO_2 treatment (GLM, *p*-value = 0.576), but a significant effect of time (GLM, *p*-value < 0.001) was detected. Productivity increased from the beginning of the experiment until t_{65} or t_{73} independently of the pCO_2 treatment (see Fig I-6), and rapidly decreased afterwards. A second increase in the productivity was detected from t_{97}, with the highest ratios in some of the high-pCO_2 mesocosms.

Regarding the *P. acuspes* females' condition, none of the physiological and reproductive parameters investigated (respiration, carbon content, prosome length, clutch size, hatching success) showed a significant difference between treatments, nor in the interaction between month and treatment (*p*-value > 0.05; Fig I-7, Table I-4). However, significant differences between the first (March, t_{19}: first phytoplankton bloom) and the second experiment (May, t_{59}: second phytoplankton bloom) were observed. Respiration rate (Fig I-7A) was lower during May compared to March (*p*-value = 0.001). Females' carbon content and prosome length, as well as the hatching success after 48h incubation (Fig I-7B, C, E) were not different between months, nor between pCO_2 conditions. Yet, at the beginning of the incubations (0h), clutch size (Fig I-7D) was significantly higher in May (*p*-value = 0.021). None of the interactions between pCO_2 treatment and month rendered in a significant effect on the studied variables.

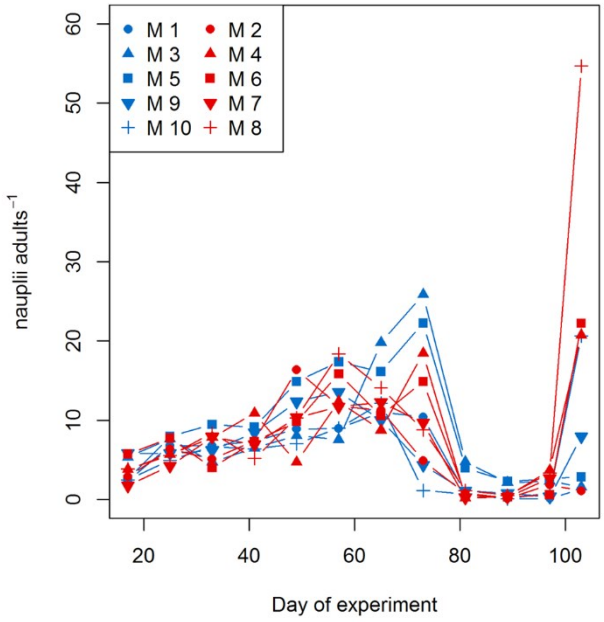

Fig I-6: *P. acuspes* productivity in relation to pCO_2 levels along the study period. Symbols and colours (blue = ambient; red = high-pCO_2 treatment) identify each mesocosm. Production estimated as the ratio between nauplii and adults. *P. acuspes* nauplii abundances were estimated from the relative abundances of *P. acuspes* in relation to total copepod abundances per sampling day and mesocosm.

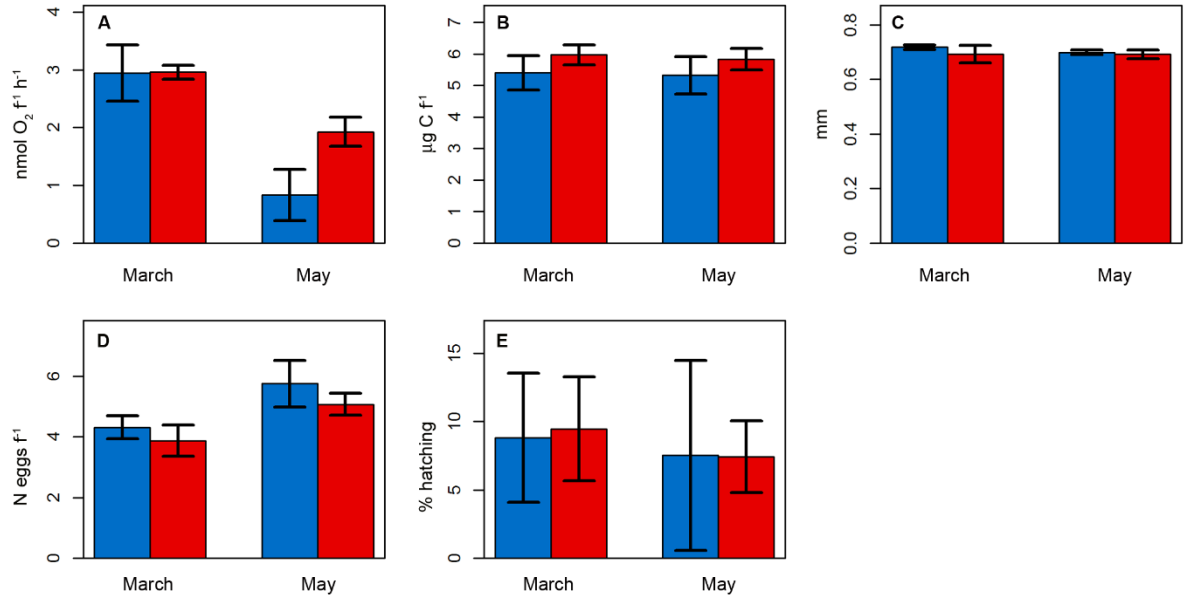

Fig I-7: *P. acuspes* females' condition. General Linear Models (GLMs) comparing the potential pCO_2 effect on *P. acuspes* females: A) respiration rate, B) carbon content, C) prosome length, D) clutch size at the beginning of the incubation (0h), E) hatching success after 48h incubation. Error bars represent

standard deviation. Colour code: red = treatment (~760 µatm pCO_2), blue = control (ambient conditions). March = t_{19} (first phytoplankton bloom), May = t_{59} (decline phase of the second phytoplankton bloom).

Table I- 4: Results from *P. acuspes* females' condition experiment. Generalized Linear Models (GLMs) based on two laboratory experiments (March, May), *n* = 120 females per experiment. Boldface represent *p*-values < 0.05.

Respiration	Estimate	Std.Error	t-value	p-value
(Intercept)	5.035	0.786	6.406	0
pCO_2 treatment	0.553	0.37	1.492	0.154
month	-0.786	0.185	-4.246	**0.001**
Carbon content				
(Intercept)	5.586	0.958	5.829	0
pCO_2 treatment	0.541	0.452	1.198	0.247
month	-0.056	0.226	-0.246	0.808
Prosome length				
(Intercept)	0.728	0.039	18.875	0
pCO_2 treatment	-0.016	0.018	-0.895	0.383
month	-0.005	0.009	-0.536	0.599
Clutch size (0h)				
(Intercept)	2.394	1.103	2.17	0.044
pCO_2 treatment	-0.563	0.52	-1.082	0.294
month	0.661	0.26	2.542	**0.021**
Hatching success				
(Intercept)	11.465	9.875	1.161	0.262
pCO_2 treatment	0.275	4.655	0.059	0.954
month	-0.823	2.328	-0.354	0.728

4. Discussion

During this winter-to-summer experiment on the effect of ocean acidification on plankton communities, we did not detect an effect of pCO_2 on either the diversity of the mesozooplankton community, nor on its development as a whole. At first sight, this may seem surprising as some taxa showed a response to OA, where others did not. The most parsimonious explanation for this apparent contradiction is the strong dominance of the copepod *P. acuspes*. As a result, changes in the relative composition of the community were

small and were not be picked up by relatively coarse indicators such as Simpson's Diversity or rank-based methods such as NMDS. Only on the last two sampling days, when *P. acuspes* abundances declined strongly, a trend towards a higher diversity under high-pCO_2 conditions became visible (Figs I-2B and I-3), and the communities under the two treatments diverged (observed also for microzooplankton (Horn et al. 2016b)). Potentially this indicates a long-term effect of high pCO_2 on the communities, but this is impossible to say as, at that time the mesocosm set-up started to deteriorate and the experiment was terminated.

Unlike previous mesocosms studies focusing on the effect of OA on natural coastal plankton communities in the Arctic (Niehoff et al. 2013) and the Baltic (Lischka et al. 2015), we detected a positive pCO_2 effect on the total mesozooplankton abundance from Gullmar Fjord. This effect was mostly caused by the CO_2-driven increase in the abundances of *P. acuspes* copepodites. This was somewhat unexpected, as previously work on the same species from the same location (Thor and Dupont 2015; Thor and Oliva 2015) found significant negative pCO_2 effects on egg production and metabolism. The two studies cited above were highly controlled laboratory experiments, where the copepods were cultured under uniform environmental conditions (except for the pCO_2 treatments) and offered identical prey in all treatments. Thus, the effects observed were directly caused by changes in carbonate chemistry of the water as all other environmental factors were identical. In semi-natural experiments such as the one described here, these effects are easily masked, either through bottom-up effects (changes in the availability or quality of the food), or as a result of top-down effects (changes in predation rates). In our two condition experiments we excluded the latter effects, and focused on the effects of the overall growing conditions in the mesocosms. In contrast to the laboratory experiments cited above, we did not find significant differences in the physiological condition of *P. acuspes* females between ambient and high-pCO_2 treatments (Fig I-7). Secondary production in *P. acuspes* followed a temporal trend, with higher clutch sizes and nauplii abundances on t_{59} (May), responding to higher phytoplankton concentration (chl*a*) and microzooplankton biomass. However, this increase in food quantity might not have been coupled with food quality to maintain the copepod population in the mesocosms, which increased from ~260 ± 5 copepods L^{-1} (t_{19}) to ~1245 ± 32 copepods L^{-1} (t_{59}). This could explain lower respiration rates in May than in March (Thor et al. 2002; Malzahn et al. 2010). Potential food items for copepods on t_{19} (March) consisted mainly of phytoplankton

between 5 and 40 µm and microzooplankton biomass below 2 µg C L^{-1} before the first phytoplankton bloom in the mesocosms (Horn et al. 2016b; Taucher et al. 2017b). On t_{59} the entire mesocosms system was dominated by *Coscinodiscus concinnus* (representing 47% of the biomass) and the nanophytoplankton fraction (accounting for 21%) (Taucher et al. 2017b), both largely outside the food spectrum of *P. acuspes*. Microzooplankton biomass was ~12 µg C L^{-1} on t_{59} (Horn et al. 2016b), but might not have been enough to supply the whole *P. acuspes* population, so copepods might have searched for alternative food sources such as sinking material. In fact, the decrease in adults from t_{97} in all mesocosms matched high resolution images taken from sediment trap material, where high abundances of adult *P. acuspes* were found (Tim Boxhammer, pers. comm.). This observation suggest that, towards the end of the experiment, copepods might have migrated downward searching for food and stayed close to the sediment traps, as previously observed in a mesocosms experiment in a Norwegian fjord (Bach et al. 2016a).

In view of the result of the two laboratory experiments, where we observed no effects of pCO_2 on egg production, the most plausible explanation for the higher *P. acuspes* abundances under the high-pCO_2 treatment is a community CO_2-driven bottom-up effect (Rossoll et al. 2012; Schoo et al. 2013; Cripps et al. 2016). This is not a contradiction, as in the laboratory experiments we specifically looked at the memory pCO_2 effect on the clutch, which was not expected to be affected by the 48h food deprivation regime (Niehoff 2003). Thus, the higher abundance of *P. acuspes* copepodites was probably fuelled by phytoplankton community responses to high-pCO_2 conditions during our mesocosms experiment. Higher primary production (Eberlein et al. 2017) and higher chl*a* levels under high-pCO_2 (Bach et al. 2016b) resulted in higher copepodite abundances. Interestingly, this CO_2-driven increase in copepodite abundances did not result in higher abundances of adults later in the season except on $t_{81,}$ when adult *P. acuspes* were significantly more abundant under high-pCO_2 conditions. The most plausible explanation for this trend in adult *P. acuspes* abundance after t_{81} is, apart from the potential downward migration as indicated above, that the level of top-down control through herring larvae was different, with higher predation pressure in high-pCO_2 mesocosms. As detailed in Sswat et al. (Sswat et al. submitted), after hatching on ~t_{63}, herring larvae would have gradually switched from endogenous to exogenous feeding, preying then firstly on nauplii and ciliates, afterwards increasing the size of their prey

gradually with their own body size until they reached copepodites (~t_{65}-t_{81}) and finally adults (~t_{81}-t_{105}) (Checkley 1982; Hufnagl and Peck 2011; Denis et al. 2016). From t_{77} (14^{th} day post-hatching, DPH) survival of herring larvae was significantly higher in the high-pCO_2 mesocosms (Sswat et al.), which would imply higher grazing pressures on *P. acuspes.* Since consumption rates of smaller larvae are much lower than those of larger ones, we would have only detected a top-down effect of the herring larvae on adult abundance at the end of the experiment. This, together with a more intensive feeding activity by herring larvae because of the higher larvae survival rates under the acidic treatment (Sswat et al.), could have caused lower abundances of adult *P. acuspes* relative to the opposite pattern in the copepodites.

In the case of *T. longicornis,* no effects of pCO_2 were observed on the adults but copepodites were more abundant under ambient conditions, especially during the last 20 days of the experiment (Table I-3, Fig I-4D to F). This finding fits to the last two sampling days divergence between treatments in the NMDS analysis (Fig I-3), which points to a different development of the community under ambient and high-pCO_2 conditions. The particular tolerance in *T. longicornis* female reproductive fitness to end-of-century pCO_2 scenarios had already been described by McConville et al. (McConville et al. 2013). However, the higher abundances of *T. longicornis* copepodites observed in ambient conditions suggest that this tolerance might be diminished in early life stages, as previously observed in other calanoid copepods (Cripps et al. 2014b; Meunier et al. 2016).

Our results suggest a negative effect of pCO_2 on adult *O. similis*, which were more abundant under ambient conditions when considering the whole experimental period. The explanation for *O. similis*' sensitivity to OA observed in adults might be in the life history of this copepod. According to Lewis et al. (Lewis et al. 2013) there is a correlation between sensitivity to OA and vertical migration behaviour. Species that do not exhibit diel vertical migration behaviour (as *O. similis*) are typically less exposed to variation in pCO_2 levels compared to other copepods and more prone to be sensitive to OA (Fitzer et al. 2012; Lewis et al. 2013). For *O. similis*, these researchers detected reduced adult and naupliar survival under 700 and 1000 µatm pCO_2. Our study would support this observation by lower *O. similis* adult abundances under high-pCO_2 conditions. Towards the end of the experiment, however, we observed an increase in *O. similis* abundance, likely reacting to the increase in ciliates and dinoflagellates biomass (Horn et al. 2016b). Adults showed a significant reaction to OA with firstly higher and

subsequently lower abundances in the high-pCO_2 treatment. As also observed on adult *P. acuspes*, the differential decrease in adult *O. similis* within treatments from t_{81} might respond to herring larvae abundance and the size-dependent feeding activity (Hufnagl and Peck 2011; Sswat et al. submitted). Thus considering that during the last two sampling days adults would probably be in the preferred size range for the herring larvae, the release in preying pressure on copepodites and the built-up of protozooplankton (Horn et al. 2016b) might explain the final increase in copepodite abundance in both treatments.

Whilst the connection between jellyfish blooms (scyphomedusae, hydromedusae, siphonophores and ctenophores) and anthropogenic climate change remains unclear (e. g. (Condon et al. 2012; Purcell 2012)), the effects of changing seawater carbonate chemistry on planktonic gelatinous species have been rarely tested. However, all results on different gelatinous zooplankton groups (schyphomedusa ephyrae (Kikkawa et al. 2010; Winans and Purcell 2010; Algueró-Muñiz et al. 2016), coelenterate records (Richardson and Gibbons 2008)) point to the tolerance of jellyfish to future changes in pCO_2. In this study we showed for the first time the species-specific sensitivity of hydromedusae to OA. Thus *H. prolifer* (Anthomedusa) reacted negatively to high pCO_2 by lower abundances, while *A. digitale* (Trachymedusa) was more abundant in the high-pCO_2 treatment (Table I-3, Fig I-5). This result was unexpected, given the fact that *A. digitale* has statoliths, which could be a target for lower pH (as Richardson and Gibbons (Richardson and Gibbons 2008) also noted). Our findings suggest that hydromedusae with statoliths are not necessarily more sensitive than those without these calcium-based structures, and consequently hydromedusa statoliths might not be sensitive to OA, at least in realistic end-of-century scenarios. Further ecophysiological analyses, however, are still required for these and other hydromedusae species to confirm this hypothesis.

Conclusion

During this study, we observed species-specific sensitivities to pCO_2 in copepods and hydromedusae abundance. In the case of copepods, responses to elevated pCO_2 depended also on the life-stage of the individuals, copepodites generally being the most sensitive stage. Our results point that OA could positively affect the calanoid *P. acuspes* by a bottom-up effect

in pCO_2-fuelled food webs. Nonetheless, the effect of OA on single species was not detectable in the structure or diversity of this community, probably due to the overwhelmingly dominance of *P. acuspes* in the studied community. Hence, under a realistic end-of-century OA scenario, the Gullmar Fjord mesozooplankton community structure is not expected to change much, although it could well be that the OA effect on copepodites would potentially affect biomass transfer to higher trophic levels in the future.

Ethic statement

No specific permission was required for activities related to field sampling. The field location was not privately owned or protected, and neither endangered nor protected species were involved. Fish larvae experiment (Sswat et al.) was conducted under the ethical permission (number 332-2012 issued by the Swedish Board of Agriculture "Jordbruksverket"). Animal welfare was assured by minimization of stress from handling and treatment. Specimens were therefore anaesthetized before handling using Tricaine methanesulfonate MS-222. The CO_2 concentrations used in this study are far below the lethal level.

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CHAPTER II

Impacts of ocean acidification on the development of a subtropical zooplankton community during oligotrophic and simulated bloom conditions

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Abstract

Ocean acidification (OA) is affecting marine ecosystems through changes in carbonate chemistry that may influence consumers, often via trophic pathways. Using a mesocosm approach, we investigated OA effects on a subtropical zooplankton community during oligotrophic, bloom, and post-bloom phases under a range of different pCO_2 levels. The pCO_2 treatments consisted of a gradient from current levels (~400 µatm) to concentrations of ~1480 µatm. Furthermore, we simulated an upwelling event by adding nutrient-rich deep water to the mesocosms, which caused a phytoplankton bloom. No effects of pCO_2 on the zooplankton community were visible in the pre-bloom situation. The zooplankton community responded to phytoplankton bloom by increased abundances in all treatments, although the response was delayed under high-pCO_2 conditions. Microzooplankton was dominated by small dinoflagellates and aloricate ciliates, which were more abundant under medium to high-pCO_2 conditions. The most abundant mesozooplankters were calanoid copepods, which did not respond to CO_2 treatments during the oligotrophic phase of the experiment, but were found in higher abundance under medium- and high-pCO_2 conditions towards the end of the experiment, most likely as a response to increases in phyto- and microzooplankton standing stocks. The second most abundant mesozooplankton taxon were Appendicularia, which did not show a response to the different pCO_2 treatments. Overall, there was a significant effect of pCO_2 on phytoplankton succession, ultimately affecting the development of the zooplankton community after the simulated upwelling event. We conclude that elevated pCO_2 may promote an increase in zooplankton abundances during phytoplankton bloom and post-bloom phases that might ultimately affect higher trophic levels in the future.

1. Introduction

Anthropogenic emissions are increasing atmospheric CO_2 concentrations from pre-industrial levels of ~280 µatm to current levels of over 400 µatm, and increases to 1000 µatm are expected by the end of the century under a RCP8.5 emission scenario (IPCC, 2013). The oceans act as carbon sinks, absorbing about one third of the anthropogenic CO_2 emission (Sabine et al., 2004), and thereby causing ocean acidification (OA). This oceanic CO_2 uptake causes a shift in carbonate chemistry with a decrease in seawater pH, commonly known as ocean acidification (OA) and may cause substantial changes to marine ecosystems (Fabry et al., 2008;IPCC, 2013).

Despite the large body of literature related to biological responses to OA, most studies investigated single species responses, which may rarely provide a sufficient basis to understand long-term responses in complex ecological environments (Harley, 2011;Queirós et al., 2015). Moreover, changes in pCO_2 may promote changes in trophic interactions, leading to the dampening or amplification of single species effects and hence promoting shifts in community composition (Lischka et al., 2011;Rossoll et al., 2012;Rossoll et al., 2013). Consequently, the combination of laboratory experiments with *in situ* mesocosm experiments is important in order to evaluate OA effects at the level of communities and ecosystems (Guinotte and Fabry, 2008;Riebesell and Gattuso, 2015).

Nutrient conditions can determine how plankton communities respond to OA (Alvarez-Fernandez et al. submitted), the most noticeable pCO_2 effects being promoted by limiting inorganic nutrient availability in different communities (Paul et al., 2015;Sala et al., 2015;Bach et al., 2016b). The present study focussed on an oligotrophic system around the island of Gran Canaria within the Canary Archipelago, located in the subtropical Northeast Atlantic Ocean. Despite its overall oligotrophic character, this region can experience short-term periods of deep-water nutrient inputs in later winter (February-March). This usually causes an increase in primary production and chlorophyll *a* concentration in the euphotic zone (Menzel and Ryther, 1961;Arístegui et al., 2001). Typically, mesozooplankton grazing pressure exerted on phytoplankton is low in the study area (Arístegui et al., 2001;Hernández-León et al., 2004), and mesozooplankters are considered to feed on microzooplankton which, in turn, control primary production (Hernández-León et al., 2001;Quevedo and Anadón, 2001;Calbet and

Alcaraz, 2007). The microzooplankton community is usually dominated by small dinoflagellates and aloricate ciliates (Quevedo and Anadón, 2001), while the most important mesozooplankton during the annual cycle are copepods (Hernández-León et al., 2007). However, the plankton community typically changes during the bloom (Arístegui et al., 2001;Hernández-León et al., 2004;Schmoker et al., 2012). An increase in copepods follows the increase in primary production, and a trophic cascade caused by the consumption of microzooplankton by mesozooplankton allows a further increase in autotrophic biomass by the combined effect of top-down control and nutrient remineralization (Hernández-León 2009; Schmoker et al. 2012). This bloom situation may cause a reduction in the efficiency of the food web, considering that trophic transfer efficiency (i.e. zooplankton growth per unit phytoplankton production) tends to be diminished under nutrient enrichment conditions (Calbet et al., 1996;Kemp et al., 2001;Calbet et al., 2014).

In order to assess the impacts of OA on zooplankton communities, we must consider not only direct effects on zooplankton caused by pH reductions, but also effects that reach consumers indirectly, through trophic pathways (Boersma et al., 2008;Rossoll et al., 2012;Cripps et al., 2016). Detrimental indirect pCO_2 effects have been described in herbivores (Schoo et al., 2013;Meunier et al., 2016) as well as in secondary consumers (Lesniowski et al., 2015). In case of copepods, bottom-up influences of OA seem to be largely associated with interspecific differences among prey items with regard to their sensitivity to elevated pCO_2 levels (Isari et al., 2015a). In turn, microzooplankton may be affected by the effect of high pCO_2 levels on phytoplankton availability or quality such as an increase in picophytoplankton standing stock or changes in carbon-to-nutrient ratios (Bach et al. 2016b; Meunier et al. 2016). Plankton community OA studies to date have been mostly carried out in relatively eutrophic environments, and lead to varying conclusions. Some studies showed tolerance to elevated pCO_2 levels in micro- (Aberle et al. 2013; Horn et al. 2016b) and mesozooplankton abundances (Niehoff et al., 2013), while others detected both changes in community size distributions (Lischka et al. 2017; Taucher et al. 2017b) and positive bottom-up pCO_2 responses on mesozooplankton abundances (Algueró-Muñiz et al. 2017). Inorganic nutrient availability would control these different responses to OA in planktonic communities, thereby the nutrient-deplete phases could determine the translation of the pCO_2 effect on primary producers to primary consumers (Alvarez-Fernandez et al. submitted). Taking this into

account, the study of OA effect in oligotrophic systems —which represent most of the ocean— becomes of paramount importance. To accomplish this goal, we present a study that allows the contrast between nutrient-repleted and nutrient-depleted periods. Our aim was to analyse the effects of OA on the development of an autumn zooplankton community from the subtropical Northeast Atlantic, including a simulated bloom situation. To do that we assessed the effects of pCO_2 on the 1) abundance of subtropical micro- and mesozooplankton under oligotrophic and upwelling conditions, 2) size and reproductive output of a poecilostomatoid copepod and 3) trophic efficiency (ratio autotrophy/heterotrophy) within the plankton community.

2. Materials & methods

2.1 Mesocosms setup and experimental design

This study was conducted from 27th September (t-4) until 26th November 2014 (t56) as part of the KOSMOS 2014 Experiment, within the framework of the BIOACID II project (Biological Impacts of Ocean ACIDification). In order to study the effects of changing carbonate chemistry conditions on the plankton community succession, nine mesocosms (KOSMOS, M1-M9: "Kiel Off-Shore Mesocosms for future Ocean Simulation"), were deployed in Gando Bay (27°55'41'' N, 15°21'55'' W), on the west coast of Gran Canaria (Canary Islands, Spain) (Taucher et al. 2017a). The nine experimental units consisted of large enclosed water volumes (~35 m^3) sealed by sediment traps installed at the bottom of each mesocosm bag. Target pCO_2 was reached at the beginning of the experiment by adding CO_2 saturated seawater to the mesocosms following the protocol described in Riebesell et al. (2013). Subsequent additions were made during the course of the experiment (days 2, 4, 6, 21 and 38) to compensate for CO_2 loss. As pCO_2 treatments we stablished a gradient from current levels to end-of-century scenarios, representing IPCC predictions for medium (RCP 6.0) and high (RCP 8.5) pCO_2 levels (IPCC, 2013). The mean pCO_2 values per mesocosms between t1 and t55 were M1=369, M2=887, M3=563, M4=716, M5=448, M7=668, M8=1025 and M9=352 µatm, respectively. Analysing the oligotrophic phase of the experiment, we observed three pCO_2 groups occurring among the mesocosms so we run a K-means cluster analysis and the outcome showed three distinguishable clusters: low-pCO_2 (M1, M9, M5; K=460 µatm) medium-pCO_2 (M3, M7, M4;

K=721 µatm) and high-pCO_2 levels (M2, M8; K=1111 µatm) (Fig II-1A) which were used for the analyses presented throughout this paper. Unfortunately, the third high-pCO_2 mesocosm (M6=976 µatm) was lost on t27 due to a storm, so data are only available until that date.

To simulate a natural upwelling event, we collected deep water (~84 m^3) from 650 m depth on t22, as described by Taucher et al. (Taucher et al. 2017a). From each mesocosm, a defined volume of water was removed from 5 m depth with a submersible pump (Grundfos SP-17-5R). Consequently, in a process of ~9 h duration during the night of t24, deep water was pumped into the mesocosms, reaching a total mesocosm volume of ~35 m^3 (see Table 1 from Taucher et al. (Taucher et al. 2017a)).

Regular sampling every 2nd day included CTD casts, water column sampling, and sediment sampling. CTD casts were carried out with a hand-held self-logging CTD probe (CTD60M, Sea and Sun Technologies) in each mesocosm and in the surrounding water. Thereby we obtained vertical profiles of temperature, salinity (Fig II-1b), pH, dissolved oxygen, chlorophyll *a*, and photosynthetically active radiation (PAR) (Taucher et al. 2017a). Water column samples were collected with "integrating water samplers" (IWS, Hydrobios, Kiel), in which a total volume of 5 L from 0-13 m depth was collected evenly through the water column. This water was either used for samples sensitive to contamination such as nutrient analyses, which were directly filled into separate containers on board, or stored in carboys for later subsampling for parameters such as phytoplankton and microzooplankton. Some analyses required larger volumes of water than could be sampled with the IWS in a reasonable time frame, e.g. pigment samples for reverse-phase high-performance liquid chromatography (HPLC) analysis. To enable a faster water collection, we used a custom-built pump system connected to a 20 L carboy. By creating a gentle vacuum and moving the inlet of the tube up and down in the mesocosm during pumping, samples similar to those from the IWS were obtained. All carboys were protected from sunlight during sampling and stored in a temperature controlled room at 16°C upon arrival on shore. Before taking subsamples from the carboys, they were carefully mixed to avoid a bias due to plankton sedimentation.

All sampling methods and analyses are described in detail by Taucher et al. (Taucher et al. 2017a) . Briefly, pigments such as Chlorophyll *a* (Chl *a* in the following) were analysed using HPLC (Fig II-1C). Nutrients (nitrate+nitrite (NOx), Fig II-1D) were measured using an

autoanalyser (SEAL Analytical, QuAAtro) coupled to an autosampler (SEAL Analytical, XY2). Phytoplankton samples for microscopy were obtained every 4 days and fixed with Lugol's solution. They were analyzed using the Utermöhl technique (Utermöhl 1958) and classified to the lowest possible taxonomical level. Biomass of phytoplankton was estimated by using conversion factors, as detailed in S1 Table (Tomas and Hasle, 1997;Ojeda, 1998;Leblanc et al., 2012).

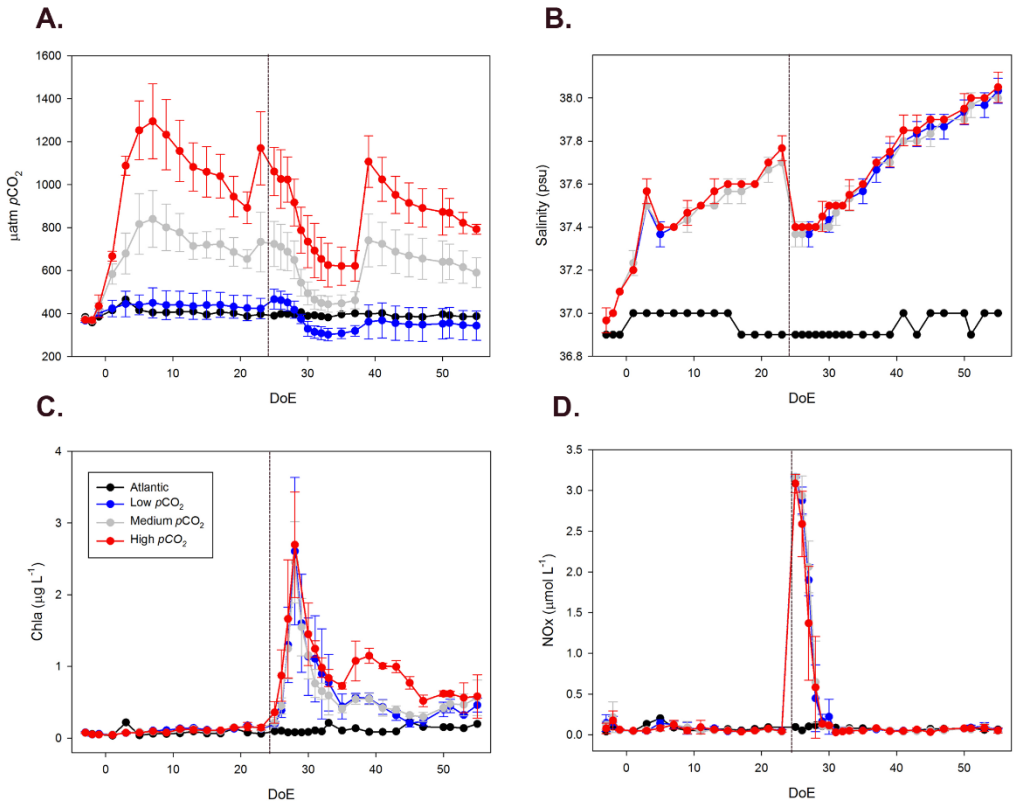

Fig II-1: Abiotic and biotic factors throughout the experiment. A) pCO_2 (µatm), B) salinity, C) Chl. *a* (µg L^{-1}), D) NOx (nitrate+nitrite; µmol L^{-1}). The addition of deep water (DW) in the mesocosms took place during the night between the 24th and 25th day of experiment (DoE); dashed line. Note that a clear draw down of CO_2 occurred during the phytoplankton bloom (t25-t35). Colour code: black = Atlantic, blue = low-pCO_2, grey = medium-pCO_2, red = high-pCO_2.

2.2 Zooplankton: sampling and analysis

For analysis of the microzooplankton community (microZP), samples from the IWS were taken every eight days, the last time point being day 50. 250 mL of mesocosm water was transferred into brown glass bottles, fixed with acidic Lugol's solution (1-2% final concentration), and

stored in the dark. MicroZP was counted and identified with an inverted microscope (Axiovert 25, Carl Zeiss) using the Utermöhl method (1958). 50 mL of each sample was transferred into a sedimentation chamber and allowed to settle for 24 h prior to counting. Depending on plankton abundances, the whole or half of the surface of the chamber was counted at 100-fold magnification to achieve a count of at least 300-400 individuals for the most common taxa. MicroZP was identified to lowest possible level (genus or species level) and otherwise grouped into size classes according to their distinct morphology. As most dinoflagellates are capable of heterotrophic feeding (Calbet and Alcaraz 2007), they can be considered as mixotrophic and were thus included in the microZP. Only few mixotrophic taxa such as *Ceratium* or *Dinophysis* are predominantly autotrophic and were thus included in the phytoplankton. MicroZP biovolumes were estimated using geometric proxies obtained from literature(Ojeda, 1998;Hillebrand et al., 1999;Montagnes et al., 2001;Schmoker et al., 2014), and transformed to carbon biomass using conversion factors provided by Putt and Stoecker (1989) and Menden-Deuer and Lessard (2000) for ciliates and dinoflagellates, respectively (see S1 Table).

The mesozooplankton community (mesoZP) was sampled in the mesocosms by vertical net hauls with an Apstein net (55 µm mesh size, 17 cm diameter) equipped with a closed cod end. Sampling depth was restricted to 13 m to avoid resuspension of the material accumulated in the sediment traps at 15 m depth. Every net haul consisted in total filtered volume of 295 L. One net haul per mesocosm was carried out once every eight days, always during the same time frame (2 to 4 pm) to avoid diel differences in community composition. Samples were rinsed on board with filtered sea water, collected in containers and brought to the on-shore laboratory (PLOCAN, ~5 nm distance), where samples were preserved in denaturated ethanol. For transportation the samples were placed in cooling boxes until fixation of the organisms.

During analysis, organisms were sorted using a stereomicroscope (Olympus SZX9) and classified until the lowest possible taxonomical level. Copepodites and adults were classified together on a species/genus level, with the exception of *Oncaea* sp., for which adults and copepodites were considered separately for a more in-depth study of this copepod. Nauplii from different species were pooled together. Taxonomical analysis was carried out focusing on copepods as the most abundant group (Boltovskoy, 1999). Every sample was sieved using

a 50 µm mesh, rinsed with tap water and divided with a Folsom plankton splitter (1:2, 1:4). Abundant species/taxa (> 200 individuals in an aliquot) were only counted from subsamples, while less abundant species/taxa were counted from the whole sample.

As a proxy to explore the system's energy transfer efficiency from producers to consumers (i.e. trophic transfer efficiency, TTE), we established the quotient autotrophy: heterotrophy (A:H) based on phytoplankton , heterotrophic microZP and mesoZP abundances transformed into biomass (see S1 Table for further details). Low efficiency (TTE) implies a smaller biomass of heterotroph per unit of autotroph, hence TTE and A:H are inversely correlated.

2.3 *Oncaea* sp. condition

Oncaea sp. is a common genus in the Canary Current System, where it has been typically recorded during the upwelling season (Hernández-León, 1998;Huskin et al., 2001;Hernández-León et al., 2007). *Oncaea* sp. is of special interest for this study because of 1) its trophic interaction with appendicularians (Go et al., 1998), which in turn may positively correlate abundances with increased pCO_2 levels and nutrient enrichment (Troedsson et al., 2013) and 2) to our knowledge, poecilostomatoid copepods had not been studied in an OA context before. Hence, despite being not the most abundant mesoZP taxon within the mesocosms (Poecilostomatoida; 8% total mesoZP catch) we focused on the condition of *Oncaea* sp. to investigate direct and/or indirect pCO_2 effects on the female copepod length and reproductive output. Females were sorted from the same samples used for species determination, i. e one sample per mesocosms (M1 to M9) every 8 days during the whole study period (see 2.2). The whole sample was scanned under the stereomicroscope (Olympus SZX9) and the first 20 adult females per sample were selected. Prosome length of every individual was measured and females were classified regarding sexual development (mature/immature) and presence or absence of the egg sack. Females with developing egg sacks were classified as mature, while females which did not present any egg sack or eggs inside were rated as immature individuals.

2.4 Statistical analyses

As an exploratory analysis, non-metric multidimensional scaling (NMDS) was used to describe the zooplankton community development per mesocosm throughout the experiment. In our case the data matrix comprised abundances of each phytoplankton, microZP and mesoZP taxon in each mesocosm and on each sampling day (69 MK_timestep x 96 taxa). The treatment effect was assessed by using permutation tests on the community position in the NMDS space. These permutations check if the area of clusters formed by the treatment in the NMDS are smaller than randomized samples of the same size (Legendre and Anderson, 1999). In a complementary approach, we applied an ANalysis Of SIMilarity (ANOSIM) test (Clarke, 1993) as a post-analysis to compare the mean of ranked dissimilarities between pCO_2 treatments to the mean of ranked dissimilarities within treatments. This analysis tests the assumption of ranges of (ranked) dissimilarities within groups are equal, or at least very similar (Buttigieg and Ramette, 2014).

To describe the temporal trends of each taxon during this experiment we used generalized additive mixed models (GAMMs) (Wood, 2006;Zuur et al., 2009) with a Gamma distribution and a logarithmic link. Three different kinds of models were fitted to each abundance group (Table II-1).

Table II-1: Generalized additive mixed model (GAMM) structures. DoE = day of experiment.

Models	Meaning
s(DoE)	temporal trend
s(DoE) : pCO₂	effect of pCO_2 on the temporal trend
s(DoE) + pCO₂	temporal trend and an independent pCO_2 effect on abundances

Each of these models allowed the abundance temporal trend to vary differently between pCO_2 treatments, representing (a) an equal temporal trend for all mesocosms (*s(DoE)*), (b) an effect of pCO_2 on the temporal trend (*s(DoE) : pCO₂*) (c) an equal temporal trend with an independent CO_2 effect (*s(DoE) + pCO₂*). This way, potential differences between pCO_2 treatments could be detected as either (b) changes in phenology or (c) an increase/decrease of overall abundance. If necessary, models were fitted with an autocorrelation structure of

first order to account for temporal autocorrelation in the data (Zuur et al., 2009). Statistically significant models were compared by the coefficient of determination (R^2), which indicates the proportion of the variance in the dependent variable that is predictable from the independent variables. For each taxon, the model with the highest R^2 was considered to best represent the abundance data. Models presented here accounted from t1, whilst t-3 abundances have been included in the figures in order to illustrate conditions prior pCO_2 manipulations within the mesocosms.

Differences in the condition of *Oncaea* females were analysed by generalized linear mixed models (GLMMs) comparing the potential effect of pCO_2 and time on development, prosome length and reproductive output. The effect of the day of experiment (t1 to t56) and pCO_2 treatment (low-, medium-, high-pCO_2) on the studied parameters as well as their interaction were considered in the models. A Poisson distribution with a log link was used for the GLM of count data, while length data was analysed with a Gamma distribution. Unfortunately, the relatively low zooplankton sampling frequency did not allow for testing pCO_2 effects on a continuous manner. As an alternative, different pCO_2 levels were grouped in low-, medium-, and high-pCO_2 according to a K-means cluster.

We used R (version 3.0.2, (Team, 2012)) to fit abundance data with the GAMMs and GLMMs. The significance level for all statistical analysis was set to $p < 0.05$.

3. Results

3.1 Community change

The 2-dimensional representation of the community showed a strong trend in time (plankton succession), and a divergence of this trend from ca. t25 between the high- pCO_2 mesocosms and the rest (Fig II-2). Treatments followed a similar trend from t-3 until t17, but tended to separate afterwards, matching the simulated upwelling caused by DW addition (t24). Permutation tests (with 999 permutations) did not show the areas (i.e. clusters of samples) representing the different pCO_2 treatments to be significantly smaller than randomized areas, indicating that the variation due to CO_2 is smaller than the variation due to time (i.e., natural succession) (ANOSIM test, *p*-value = 0.246). Areas representing the sampling day were

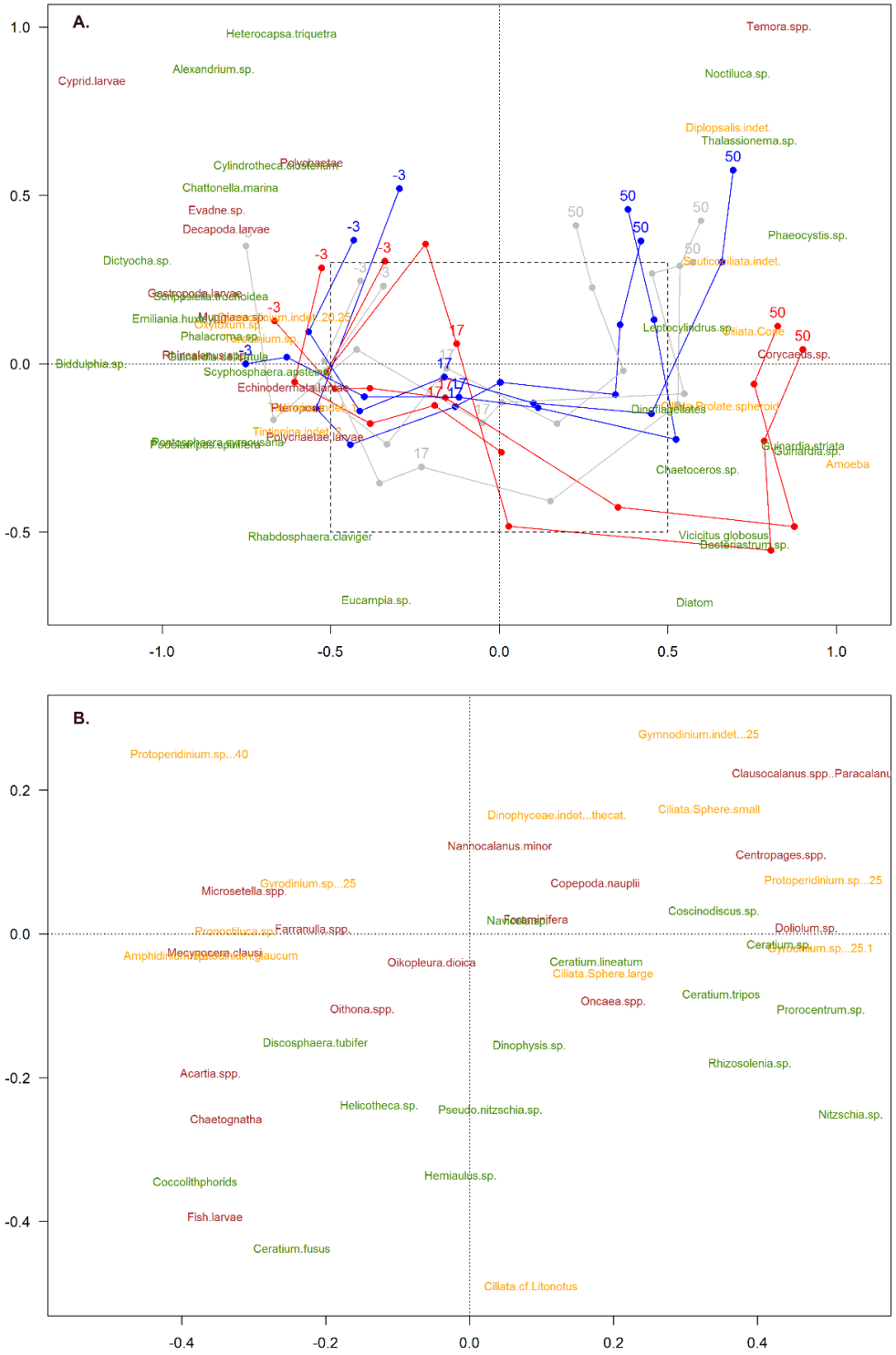

Fig II-2: Non-metric Multidimensional Scaling analysis (NMDS) of the plankton community (stress value = 0.18). Colour code: blue = low-pCO_2 (M1, M5, M9), grey = medium-pCO_2 (M3, M4, M7), red = high-pCO_2 (M2, M6, M8). Only common species (> 0.5% total abundances) represented. Taxa names: phytoplankton (green), microzooplankton (yellow), mesozooplankton (burgundy). The numbers -3, 17 and 51 indicate sampling days; lines represent patterns. Days of experiment included in the NMDS analysis were limited to t50, due to the lack of microZP samples from t56. Amplified area (B) is a zoom-in for a clearer view of the species that overlapped in the middle of the first graph (not shown in (A) for the sake of clarity).

significantly different from randomized areas using the same test, indicating a temporal trend (*p*-value = 0.001). Moreover, results for the interaction between sampling day and pCO_2 treatment (ANOSIM test, *p*-value = 0.001) matched with the NMDS, suggesting that there was a significant effect of pCO_2 on plankton succession, ultimately affecting the development of the plankton community after the simulated upwelling event. Consequently, plankton community developed differently within the different pCO_2 treatments.

3.2 Abundance temporal trends

In view of zooplankton abundance and Chl *a* levels (Fig II-1C, (Taucher et al. 2017a)) we could define three experimental phases: pre-bloom (from t1 until DW addition on t24), bloom (t25 to 35) and post-bloom phase (from t35 until the end of the experiment).

The microzooplankton (microZP) community comprised 13 different taxonomic groups of heterotrophic dinoflagellates and ciliates. Temporal trends of total microZP were affected by pCO_2 (*s(DoE):Treat*, Table II-2), resulting in higher abundances under the high-pCO_2 treatment on the last sampling day. Averaged microZP abundances at the beginning of the experiment (t1) were $4.5 \cdot 10^6 \pm 2.89 \cdot 10^6$ individuals per m^3 for the low-, $3.45 \cdot 10^6 \pm 8.03 \cdot 10^5$ for the medium-, and $4.07 \cdot 10^6 \pm 9.36 \cdot 10^5$ for the high-pCO_2 treatments, respectively. After DW addition (t24), abundances increased in all treatments, reaching maximum abundances at the end of the experiment (t50) with $1.44 \cdot 10^7 \pm 6.61 \cdot 10^6$ individuals per m^3 in the low, $1.52 \cdot 10^7 \pm 1.08 \cdot 10^7$ in the medium, and $2.14 \cdot 10^7 \pm 8.94 \cdot 10^6$ in the high pCO_2 treatments. Microzooplankton responded rapidly to phytoplankton bloom formation following the simulated upwelling (t24) and showed the strongest increase in abundance in the medium-pCO_2 treatment. On t50, however, abundances in the medium-pCO_2 treatment decreased again while a pronounced increase in the high-pCO_2 was observed (Fig II-3G).

Microzooplankton were grouped into ciliates (aloricate and loricate) and dinoflagellates (athecate and thecate, size classes: small (<25 µm) and large (>25 µm)) for a better understanding of each group's role within the mesocosms plankton community. Aloricate ciliates, mainly represented by spherical ciliates <30 µm, accounted for ~26 % on average of total microZP abundances. They increased in abundance after t35, matching with Chl *a*

decrease (Fig II-1). An effect of pCO_2 on the temporal trend was detected on these ciliate abundances (*s(DoE):Treat*), resulting in a direct link between CO_2-enhanced phytoplankton growth and increases in ciliate abundance under high-pCO_2 conditions (Table II-2, Fig II-3A). Aloricate ciliates were clearly dominant while loricate ciliates, mainly represented by small tintinnids, accounted for only ~2.5 % of total microZP catch. No significant pCO_2 effect was detected on the temporal trend of loricate ciliates (*s(DoE)+Treat*), even though abundances were higher at lower pCO_2 during the pre-bloom phase of the experiment (Table II-2, Fig II-3B). Most dinoflagellates in low-and medium-pCO_2 treatments responded to the DW addition and followed the Chl *a* built-up and decrease (Fig II-1) resulting in an increase in dinoflagellates abundance following DW addition (t24), although only some (>25 µm athecate) responded to high-pCO_2 at the end of the experiment (Fig II-3C-F). Small athecate dinoflagellates abundance (Fig II-3C) was higher under high-pCO_2 conditions during most of the pre-bloom phase, although highest abundances were recorded under medium-pCO_2 treatment towards the end of the experiment (*s(DoE):Treat*). The most abundant group within the dinoflagellates were small thecate dinoflagellates. The best fitting model was an interaction of pCO_2 and the temporal trend resulting in higher abundances at medium pCO_2 in the second half of the experiment (*s(DoE):Treat*). Thus higher abundances of this group were recorded at medium- and low-pCO_2 treatments during the bloom, followed by a subsequent decrease in the post-bloom phase (Table II-2, Fig II-3D). Large athecate dinoflagellates (Fig II-3E) showed a similar trend during the bloom phase, but abundance resulted to be ultimately higher under low-pCO_2 towards the end of the experiment (*s(DoE):Treat*). Large thecate dinoflagellates (Fig II-3F) responded differently than other dinoflagellates, reaching lowest abundance before DW addition and increasing again when the phytoplankton bloom decayed, independent of the pCO_2 treatment (*s(DoE)+Treat*). Large dinoflagellates were mainly represented by the genus *Gyrodinium*, comprising ~12% of the total microZP abundances. Small dinoflagellates from the genera *Protoperidinium* and *Gymnodinium* accounted for ~22 and 20% total microZP abundances, respectively.

The mesozooplankton (mesoZP) community was dominated by copepods, and comprised 28 different species or taxonomic groups (see Table II-3). Nauplii were counted from the net hauls (>55 µm) and were accordingly included into mesoZP category. Total mesoZP catch showed a different temporal trend for each pCO_2 treatment (*s(DoE):Treat*, Table II-2).

Averaged mesoZP abundances at the beginning of the experiment (t1) varied between 4730 ± 1202 (low-pCO_2), 6023 ± 982 (medium-pCO_2) and 5242 ± 369 (high-pCO_2) individuals per m^3, respectively. On the last sampling day, averaged abundances were highest for the three treatments: 23038 ± 9230 individuals per m^3 in low-pCO_2, 25295 ± 14196 in medium-pCO_2 and 24403 ± 10928 in high-pCO_2, respectively. In summary, our results showed that mesozooplankton abundances increased after DW addition (t24), recording highest abundances for the three treatments on the last sampling day (Fig II-4).

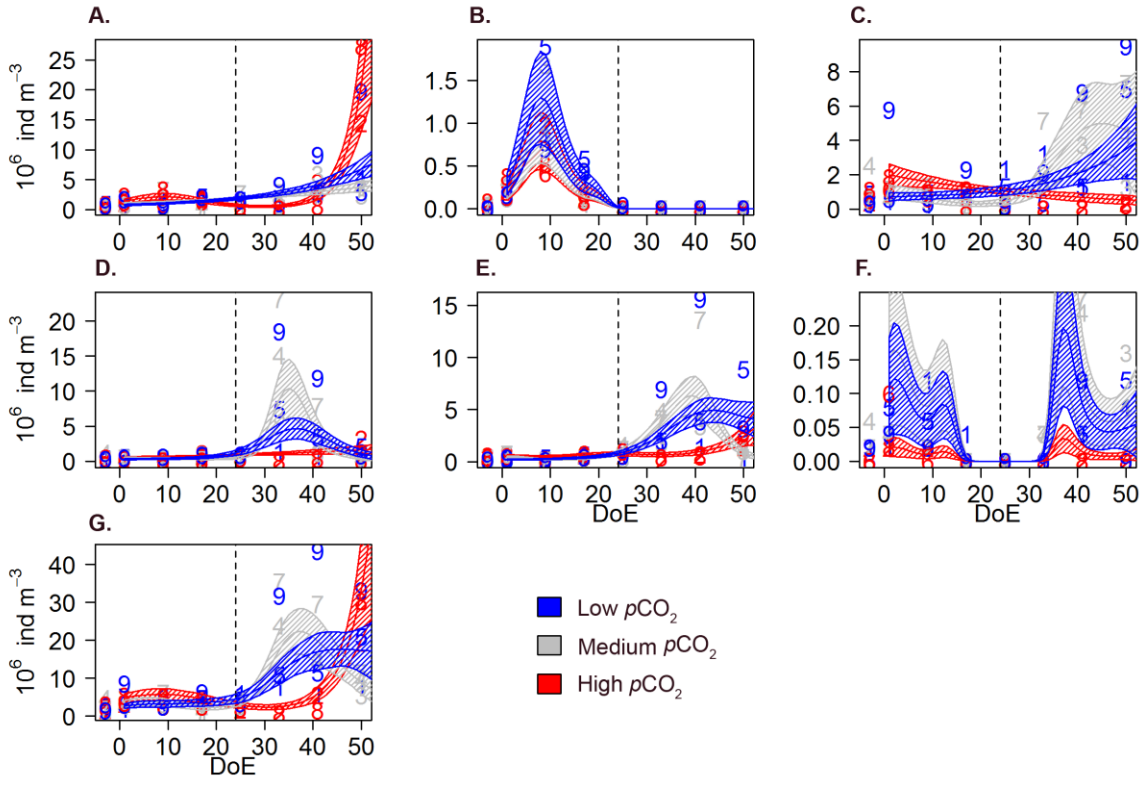

Fig II-3: Microzooplankton abundances during the study period. A) aloricate ciliates, B) loricate ciliates, C) small athecate dinoflagellates (< 25 µm), D) small thecate dinoflagellates (< 25 µm), E) large athecate dinoflagellates (> 25 µm), F) large thecate dinoflagellates (> 25 µm), G) total microZP. Colour code: blue = low-pCO_2 (M1, M5, M9), grey = medium-pCO_2 (M3, M4, M7), red = high-pCO_2 (M2, M6, M8). DoE: day of experiment. Note that, for a better visibility of the data, y-axes have been adapted to abundances in each panel. Numbers represent abundances per mesocosm (M). Solid lines = prediction from Generalized Additive Mixed Models (GAMMs) (smoother trends *p*-value < 0.05); shaded area = confidence interval. Dashed line: t24, deep water addition.

Table II-2: Zooplankton GAMM analyses. Models defined the temporal trend of the abundances alone (*s(DoE)*), or within an interaction with the pCO_2 treatments (*s(DoE):Treat*). Only significant values (*p*-value < 0.05) are presented. DoE = day of experiment; edf = estimated degrees of freedom. Significance codes: <0.001 '***' 0.001 '**' 0.01 '*' 0.05.

MICROZOOPLANKTON	Model	edf	F		R^2-adj.	Dev. Expl. (%)
aloricate ciliates	*s(DoE):Treat*	4.106	11.26	***	0.69	72.6
loricate ciliates	*s(DoE)+Treat*	6.779	579.2	***	0.753	79
athec dinoflag. <25 µm	*s(DoE):Treat*	4.035	3.287	*	0.38	39.3
thec dinoflag. <25 µm	*s(DoE):Treat*	5.219	7.227	***	0.438	55.1
athec dinoflag. >25 µm	*s(DoE):Treat*	5.388	13.191	***	0.385	79.7
thec dinoflag. >25 µm	*s(DoE)+Treat*	6.886	91.33	***	0.113	32.2
total microZP	*s(DoE):Treat*	3.568	6.259	*	0.488	42.3
MESOZOOPLANKTON						
Calanoida	*s(DoE):Treat*	3.062	37.07	***	0.726	81.4
Cyclopoida	*s(DoE)*	6.275	19	***	0.289	36.7
Harpacticoida	*s(DoE)*	1	87.91	***	0.756	37.9
Poecilostomatoida	*s(DoE):Treat*	5.95	7.664	***	0.382	37.4
nauplii	*s(DoE):Treat*	1.372	5.912	**	0.329	40.6
O. dioica	*s(DoE)*	5.739	3.98	**	0.151	13.6
mesoZP total catch	*s(DoE):Treat*	3.596	5.786	***	0.571	67.1
Oncaea sp.						
Adults	*s(DoE):Treat*	2.144	7.533	**	0.204	9.37
Copepodites	*s(DoE):Treat*	2.062	5.914	***	0.146	17.2

Different responses to pCO_2 treatments were observed among the studied copepod orders. All copepods, including nauplii, represented ~90% of total mesozooplankton abundances. Calanoid copepods were mainly represented by *Clausocalanus* spp. and *Paracalanus* spp. (including e.g. *C. furcatus, C. arcuicornis, P. indicus*), and accounted for ~46% of the total mesozooplankton abundances during the present study. An increase in calanoid abundances was detected after DW addition (t24) in low- and medium-pCO_2. Calanoida evolved similarly within the low- and the medium-pCO_2 treatments until ~t40, when abundances under medium-pCO_2 and high-pCO_2 treatments increased, resulting in abundances higher than

those in low-pCO_2 mesocosms at the last sampling day (Fig II-4A). Hence, a significant interaction between pCO_2 and temporal trend abundances was detected on calanoid abundances (*s(DoE):Treat*, Table II-2) resulting in higher abundances under elevated pCO_2 conditions (medium- and high-) during the last two sampling days.

Table II-3: Complete list of mesozooplankton species and taxa present in the mesocosms registered throughout the study period.

1	Foraminifera	15	*Farranulla* sp.
2	Hydromedusae	16	*Mecynocera clausi*
3	*Muggiaea* sp.	17	*Microsetella* sp.
4	*Doliolum* sp.	18	*Nannocalanus minor*
5	Gastropoda larvae	19	*Oithona* spp.
6	Pteropoda	20	*Oncaea* sp.
7	Polychaetae larvae	21	*Rhincalanus* sp.
8	Polychaetae	22	*Temora* sp.
9	*Evadne* sp.	23	Chaetognatha
10	Copepoda nauplii	24	Cyprid larvae
11	*Acartia* sp.	25	Decapoda larvae
12	*Centropages* sp.	26	Echinodermata larvae
13	*Clausocalanus* spp./*Paracalanus* spp.	27	*Oikopleura dioica*
14	*Corycaeus* sp.	28	Fish larvae

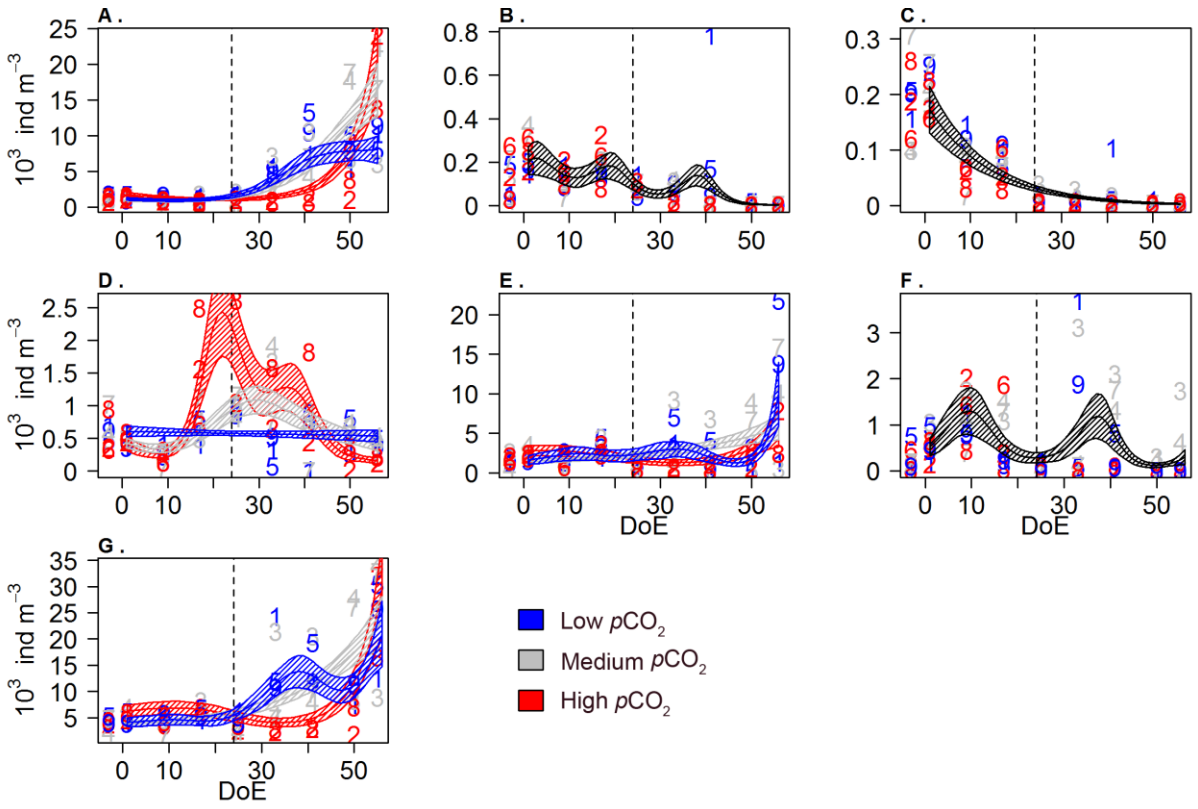

Fig II-4: Mesozooplankton abundances during the study period. A) Calanoida, B) Cyclopoida, C) Harpacticoida, D) Poecilostomatoida, E) copepod nauplii, F) *O. dioica*, G) mesoZP total catch. Colour code: blue = low-pCO_2 (M1, M5, M9), grey = medium-pCO_2 (M3, M4, M7), red = high-pCO_2 (M2, M6, M8). Note that the black lines indicate that the model prediction for the three treatments is the same. DoE: day of experiment. For a better visibility of the data, y-axes have been adapted to abundances in each panel. Numbers represent abundances per mesocosm (M). Solid lines = prediction from Generalized Additive Mixed Models (GAMMs) (smoother trends *p*-value < 0.05); shaded area = confidence interval. Dashed line: t24, deep water addition.

Cyclopoid copepods abundance (Fig II-4B), decreased throughout the experiment, independent of the treatment (*s(DoE)*, Table II-2). This order of copepods was mainly represented by *Oithona* sp. Harpacticoid copepod abundances (Fig II-4C) decreased from the start of the experiment, and no pCO_2 effect was detected (*s(DoE)*, Table II-2). This order of copepods was only represented by *Microsetella* sp. during this experiment. A significant effect of pCO_2 on the temporal trend was detected on poecilostomatoid copepods (Fig II-4D), mainly represented by *Oncaea* sp. (*s(DoE):Treat*, Table II-2). Poecilostomatiods abundance was highest in high-pCO_2, increasing until ~t25 and decreasing gradually afterwards until the end of the experiment. A similar trend was observed under medium-pCO_2 while abundances

under low-pCO_2 conditions did not vary much during the experiment. pCO_2 had an effect on the temporal trend of nauplii abundances (*s(DoE):Treat*, Table II-2), which accounted for ~33% of total mesozooplankton abundances. An increase in nauplii abundances under low- and medium-pCO_2 conditions was detected after the DW addition (t24), with maximum abundances under the medium-pCO_2 treatment (Fig II-4E), while at high-pCO_2 abundances did not increase until the last sampling day.

O. dioica population was mainly composed by juveniles, and accounted for ~6% of total mesozooplankton catch. Our analysis could not detect a pCO_2 effect on *O. dioica* during the experiment, even though they were completely absent in the high-pCO_2 treatment after DW addition (*s(DoE)*, Table II-2, Fig II-4F). This lack of detection could be attributed to the strong within treatment variability.

3.3 *Oncaea* sp.

A significant effect of pCO_2 on the temporal trend was detected on both adults and copepodites (*s(DoE):Treat*), although no reaction to DW addition (t24) was observed. Elevated pCO_2 levels resulted in higher abundances for both adults (only under high-pCO_2) and copepodites (under both medium- and high-pCO_2 conditions) (Fig II-5, Table II-2).

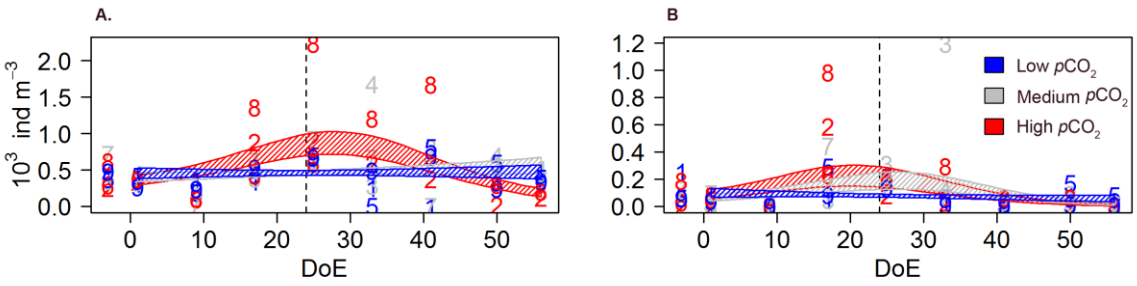

Fig II-5: *Oncaea* sp. abundances during the study period. A) adults, B) copepodites. Colour code: blue = low-pCO_2 (M1, M5, M9), grey = medium-pCO_2 (M3, M4, M7), red = high-pCO_2 (M2, M6, M8). DoE: day of experiment. Numbers represent abundances per mesocosm (M). Solid lines = prediction from Generalized Additive Mixed Models (GAMMs) (smoother trends *p*-value < 0.05); shaded area = confidence interval. Dashed line: t24, deep water addition.

A GLMM detected a negative pCO_2 effect on females' sexual development, resulting in higher number of immature females under high- pCO_2 conditions (*s(DoE):Treat.,* Table II-2, Fig II-6). Approximately 60% of the females in the high-pCO_2 mesocosms were classified as immature, versus ~30% in medium- and ~36% low-pCO_2 treatments. The number of immature females at high and low-pCO_2 increased during the experiment while it decreased under medium-pCO_2 (Fig II-6A). There were no apparent differences between the numbers of mature females without eggs across treatments (Fig II-6B). Oppositely, the number of females carrying eggs during the experiment was significantly different across treatments. At high-pCO_2 there were no egg-carrying females after t24, and a clear increase in numbers could only be detected at medium-pCO_2 (Fig II-6C). Thus, a clear negative effect at high-pCO_2 on *Oncaea* potential offspring (Table II-2, Fig II-6), represented by females carrying an egg-sac was observed.

Table II-4: *Oncaea* females' condition. Summary of GLMMs on mature and immature individuals (*n* = 20 females per mesocosms). Models (GLMMs) defined the pCO2 effect in time of *Oncaea* sp. females development and offspring *DoE:Treat*. DoE = day of experiment; edf = estimated degrees of freedom. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05.

Oncaea sp. females	Model	edf	Null deviance	p-value	pseudo-R^2
Nr. immature females	*DoE:Treat*	5	226.62	**	0.620
Nr of egg-carrying females	*DoE:Treat:egg sac*	11	6.769	***	0.598
Length of females (immature)	*DoE:Treat*	5	17.97	**	0.065
Length of females (mature)	*s(DoE):Treat:eggs*	11	19.585	***	0.104

Concerning females' prosome length (Fig II-7), the model showed a negative effect of the pCO_2 treatment on *Oncaea* sp. mature and immature females (Table II-4), although this result must be taken with caution due to the low fit of our models (pseudo-R^2 ~0.1, Table II-4). Pooling together mature and immature individuals, females prosome length was slightly shorter under high-pCO_2 conditions (0.45 ± 0.058 mm) when compared to medium-pCO_2 (0.56 ± 0.085 mm) and low-pCO_2 (0.52 ± 0.082 mm). Mature females were observed to be generally bigger than immature females during the experiment.

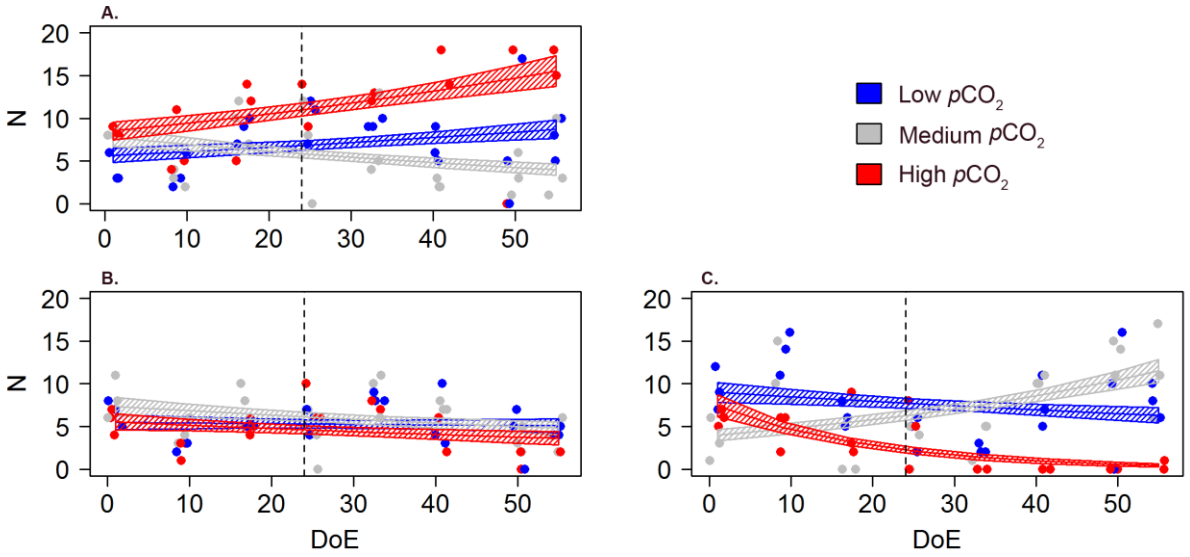

Fig II-6: pCO_2 effect on Oncaea sp. females' development and offspring (N). A) number of immature females, B) number of mature females (no egg sac), C) number of egg-carrying females. Colour code: blue = low-pCO_2 (M1, M5, M9), grey = medium-pCO_2 (M3, M4, M7), red = high-pCO_2 (M2, M6, M8). DoE: day of experiment. Solid lines = GLMM predictions (p-value > 0.05). Dashed area = GLMM predictions confidence interval.

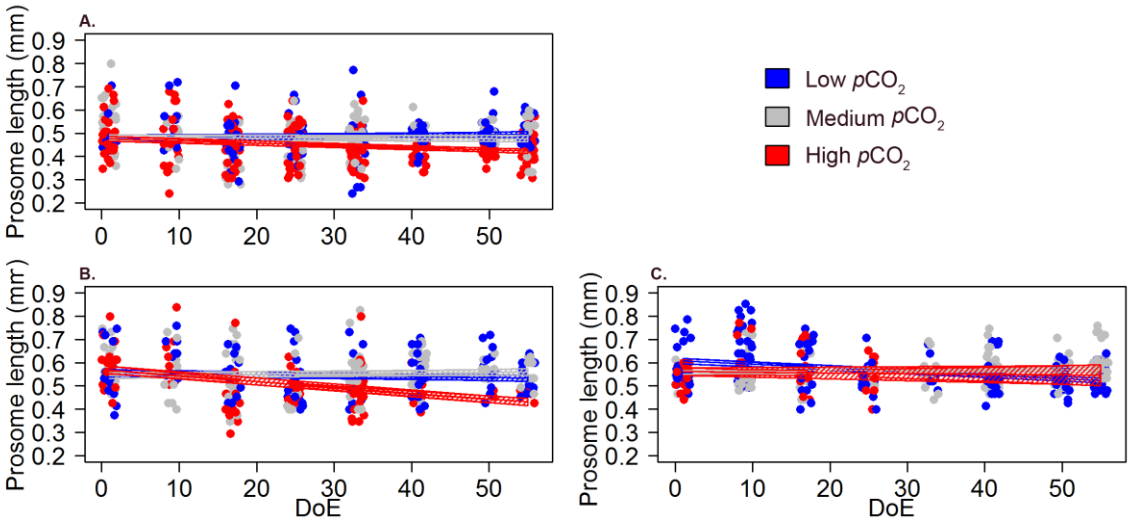

Fig II-7: pCO_2 effect on Oncaea sp. females' development and offspring (length). A) length of immature females, B) length of mature females (no egg-sac), C) length of egg-carrying females. Colour code: blue = low-pCO_2 (M1, M5, M9), grey = medium-pCO_2 (M3, M4, M7), red = high- pCO_2 (M2, M6, M8). DoE: day of experiment. Solid lines = GLMM predictions (p-value > 0.05). Dashed area = GLMM predictions confidence interval.

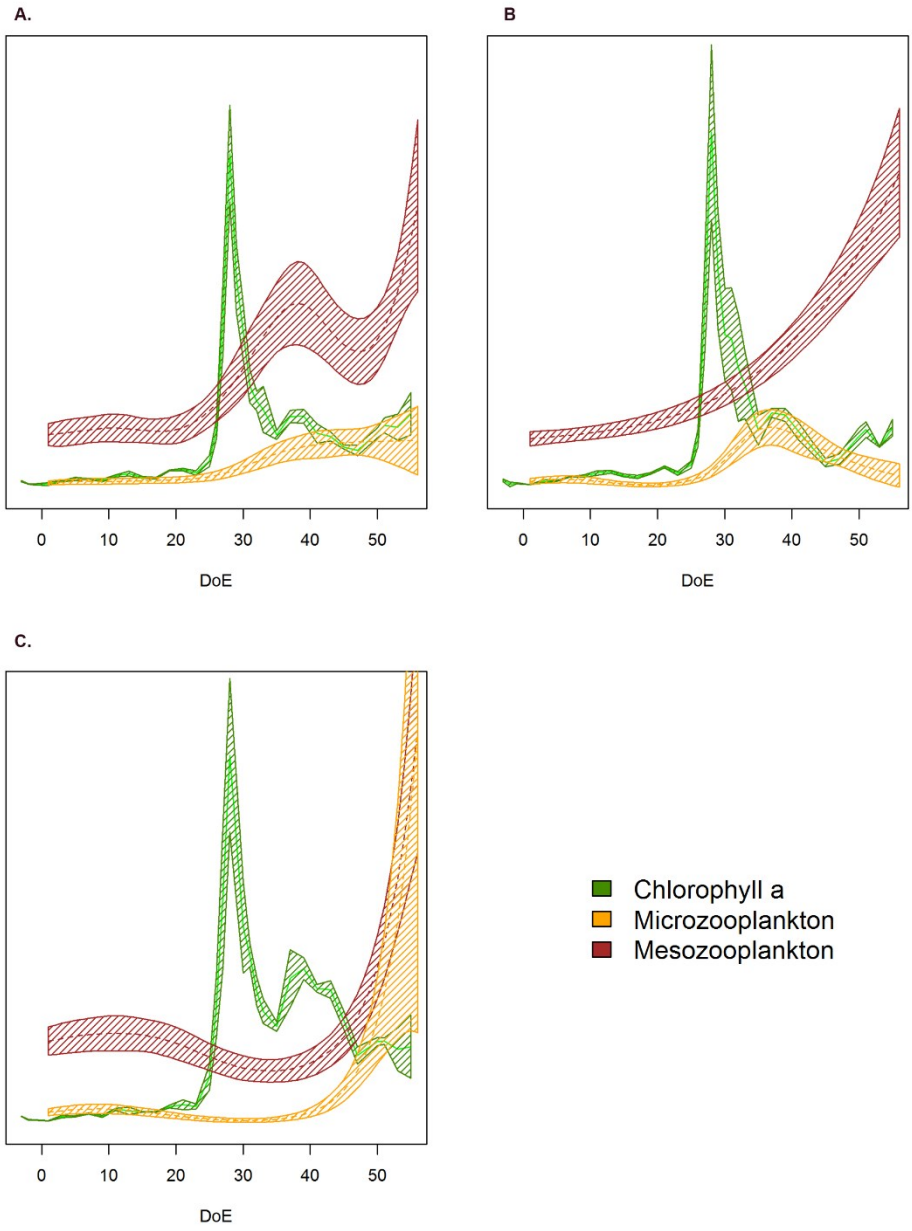

Fig II-8: Plankton succession trends. A) Low-pCO_2 treatment, B) medium- pCO_2 treatment, C) high-pCO_2 treatment. Note that trends have been transformed to be in a 0 to 1 scale to enhance plankton succession visibility. Colour code: green = Chl *a*, yellow = microZP abundance, burgundy = mesoZP abundance. DoE: day of experiment. Solid lines = prediction from Generalized Additive Mixed Models (GAMMs) (smoother trends p-value < 0.05); shaded area = confidence interval.

3.5 Trophic transfer efficiency (TTE)

The simulated upwelling caused a phytoplankton bloom (t25-t35) and subsequent pronounced differences in succession patterns and food-web structure under high CO_2 conditions (Fig II-8). There was a second and smaller phytoplankton bloom in the high-pCO_2

mesocosms (Fig II-8C) dominated by *Vicicitus globosus* (Dictyochophyceae), identified by Riebesell et al. (Riebesell et al., in prep). Harmful or non-edible for zooplankton, it seems likely that the abundance of *V. globosus* caused adverse effects on the plankton community (Chang, 2015) thus preventing the phytoplankton standing stock to reach consumers in the high-pCO_2 mesocosms until the bloom decayed (~t48). These different phytoplankton situations depending on the pCO_2 treatment were in turn reflected by changes in zooplankton community development during the second half of the experiment. Thus, while microZP abundance boosted only in high-pCO_2 treatment, we observed an increase in mesoZP abundances in both medium- and high-pCO_2 conditions towards the end of the experiment.

GAMMs showed a significant pCO_2 effect on the temporal trend of the A:H ratio (*s(DoE):Treat*, p-value < 0.05, Fig II-9). The model detected lowest TTE (higher A:H) at the end of the phytoplankton bloom (t25-t35) in the high-pCO_2 treatment. During the post-bloom phase (i.e. after t35), the A:H ratio responded to the differential increase in microZP and mesoZP abundances (see Fig II-3G and Fig I-4G). Hence A:H in high-pCO_2 decreased faster than in the other two treatments, overlapping ambient A:H on t50, when highest values corresponded to medium-pCO_2 treatment.

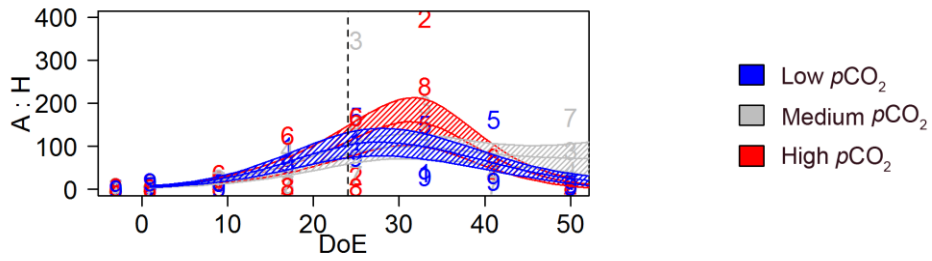

Fig II-9: Trophic transfer efficiency; autotrophy versus heterotrophy (A:H). Autotroph:heterotroph biomass ratio based on biomass estimations (µg C L^{-1}). Colour code: blue = low-pCO_2 (M1, M5, M9), grey = medium-pCO_2 (M3, M4, M7), red = high-pCO_2 (M2, M6, M8). DoE: day of experiment. Solid lines = prediction from Generalized Additive Mixed Models (GAMMs) (smoother trends p-value < 0.05); shaded area = confidence interval. Dashed line: t24, deep water addition.

4. Discussion

The main objective of this study was to analyse the effect of OA on zooplankton community from typically oligotrophic waters during pre-bloom, bloom and post-bloom conditions.

During the pre-bloom phase of this experiment we could not detect major differences between treatments on total zooplankton abundance (Figs II-3G and II-4G). However, after the simulated upwelling, the plankton community development under high-pCO_2 conditions evolved differently from the low- and medium-pCO_2 mesocosms (Fig II-2), highlighting the role that nutrient conditions play in zooplankton response to OA (Alvarez-Fernandez et al. submitted). Zooplankton abundance (Figs II-3G and II-4G) under high-pCO_2 built up much later in the experiment than those from medium- and low-pCO_2 treatments. Overall, higher zooplankton abundances (copepods, ciliates, dinoflagellates) were observed at elevated pCO_2 conditions (medium- and high-) in the post-bloom phase. This result matches with a previous mesocosm study in Gullmar Fjord (Bach et al., 2016) where a pCO_2-fuelled autotroph community can promote a bottom-up effect on certain groups of consumers, resulting in higher zooplankton abundances under moderate IPCC end-of-century pCO_2 scenarios (RCP6.0) (Horn et al. 2016b; Algueró-Muñiz et al. 2017).

4.1 pCO_2 effects on ciliates and dinoflagellates

The initial microZP abundance, as well as the taxonomic composition, agreed with previous studies in this area (Ojeda, 1998;Schmoker et al., 2014). Especially during the post-bloom phase, microZP in this subtropical area was dominated by dinoflagellates <25 µm and aloricate ciliates. In general, ciliates and dinoflagellates are considered as the main grazers in oligotrophic systems, and they also contribute to a large part of copepod diets (Calbet, 2008). This is related both to the size and nutritional quality of microZP in comparison to phytoplankton (Stoecker and Capuzzo, 1990) and the dominance of small-sized phytoplankton in oligotrophic systems which is considered as inedible for larger mesozooplankton (Kleppel 1993). In contrast to a system dominated by picoplanktonic *Synechococcus* during the pre-bloom phase, the phytoplankton bloom following the simulated upwelling was dominated by large, chain-forming diatoms (Taucher et al. 2017a). They are considered as an ideal food source for larger mesoZP and this direct consumption of mesoZP on phytoplankton might have caused a release of microZP from grazing pressure at medium- and low-pCO_2 conditions.

Previous OA studies on plankton communities mostly reported on a tolerance of microzooplankton towards high CO_2 concentrations, or only subtle changes in the community (Suffrian et al. 2008; Aberle et al. 2013; Horn et al. 2016b; Lischka et al. 2017) while other studies showed some detrimental (Calbet et al., 2014) or positive effects (Rose et al., 2009). Even though some pH sensitivity of ciliates has been shown under pH values of 6.0 (Nielsen et al., 2010), ciliates are in general considered as rather robust towards direct effects of pH. In contrast, tintinnids started decreasing after t10 and were virtually absent after DW addition. An increase in aloricate ciliates abundance was observed in all treatments in response to the DW-induced phytoplankton bloom, although the increase showed a considerable time-lag in relation to increases in phytoplankton standing stocks, especially at high CO_2 conditions. Such a delayed response of aloricate ciliates to increases in phytoplankton availability is considered as rather unusual (Smetacek 1981; Johansson et al. 2004; Aberle et al. 2007) and a potential explanation for this could be related to (1) inadequate food sources (*V. globosus*) or (2) top-down control by copepods. In contrast to aloricate ciliates, loricate ciliates showed only a (very small) peak during the pre-bloom phase, starting to decrease after t10 and absence after DW addition. For dinoflagellates, especially small-sized athecate ones, a positive effect of high CO_2 levels was expected based on previous OA studies conducted in oligotrophic (Sala et al., 2016) and eutrophic areas (Horn et al. 2016b). During the pre-bloom phase of the experiment, this assumption was confirmed since higher abundances of small athecate dinoflagellates at high CO_2 were observed. Unlike ciliates, heterotrophic dinoflagellates are known to feed on phytoplankton of various sizes up to several times larger than their body size and have been shown to prey on bloom-forming diatoms including taxa as e.g. *Thalassiosira* (Sherr and Sherr, 2007). The abundance of diatoms, however, was lower at high-pCO_2 compared to the low- and medium-pCO_2 conditions thus the effect of a high-pCO_2 on dinoflagellates was most likely an indirect one based on changes in the phytoplankton composition.

4.2 pCO_2 effects on plankton succession

The lack or delay in the response of ciliates and dinoflagellates to the simulated upwelling in high-pCO_2 mesocosms (M2, M8) might have been caused by the potentially harmful algae (*V.*

globosus, Riebesell et al. in prep.), which bloomed only in the high-pCO_2 mesocosms from t35 until t47. Although no linear correlation was found between *V. globosus* and ciliates, dinoflagellates, copepod or nauplii abundances (Pearson correlation, p-value > 0.05), the expected responses of microZP to increases in phytoplankton availability under nutrient-rich high-pCO_2 conditions was only detected when the second phytoplankton bloom decayed. Hence, microZP as potential grazers were most likely affected by the inadequacy of *V. globosus* as food (Chang 2015) , thus preventing the subsequent increase in mesoZP abundances via bottom-up control. This is even more likely considering that once the *V. globosus* bloom ceased, microZP started to increase in numbers in the high-pCO_2 treatments as well at a time point when they were already decreasing at low and medium-pCO_2. The tolerance to harmful algae has been previously described for copepod species close to those recorded in the mesocosms such as *Paracalanus parvus* (tolerant to *Chatonella antiqua*) and *Oncaea venusta* (tolerant to *Karenia brevis*) (Turner and Tester, 1989). Although *Paracalanus* sp. nauplii may exhibit adverse effects from feeding upon *Alexandrium tamiyavanichii* (Silva et al., 2013), we have not detected negative effects on nauplii abundances when relating them to *V. globosus*, but a delay in the reaction time likewise in aloricate ciliates and calanoid copepods. Accordingly, we based our conclusions for copepods on temporal trends and pCO_2 treatments rather than on possible harmful effects. Our results suggest that copepods reacted to the different pCO_2 levels only after their preferred prey (i. e. heterotrophic protists (Turner, 2004)) reacted to the simulated bloom, thus highlighting the importance of microZP in bloom situations within oligotrophic ecosystems (Calbet and Alcaraz 2007; Calbet 2008).

4.3 Bottom-up control on mesozooplankton community

As reported by other authors (Isari et al., 2015b), copepod response to OA is not only species-specific, but also depends on the community and the trophic interactions that can be established. Accordingly, our results revealed different sensitivities of the studied copepods from this oligotrophic system, as well as the amplification of the pCO_2 effects after the simulated upwelling event. The temporal trends in major microZP groups (aloricate ciliates, small dinoflagellates) and Calanoida (Fig II-3 and II-4) are most likely explained by the food supply for microZP and a preference for heterotrophic protists in the diets of calanoids (Suzuki

et al., 1999;Turner, 2004) during the present study. The different life stages of copepods might be indirectly affected by pCO_2 when feeding on phytoplankton or on grazers (Turner 2004), ultimately reinforcing the influence of CO_2-driven phytoplankton boost on mesoZP community (Rossoll et al. 2012; Algueró-Muñiz et al. 2017; Taucher et al. 2017b). Calanoida resulted to be positively affected by medium- and high-pCO_2, although the trend was only visible during the last two sampling days. These results match with previous ones described for copepodites and adult *Pseudocalanus acuspes* in eutrophic waters and pCO_2 levels of ~760 µatm (Algueró-Muñiz et al. 2017; Taucher et al. 2017a), suggesting a benefit of realistic end-of-century pCO_2 levels on calanoid copepods within pCO_2-fuelled communities. The delay in the response of calanoid copepods to the simulated upwelling under high-pCO_2 treatment can be explained by detrimental direct and/or indirect effects of the *V. globosus* bloom (Riebesell et al., in prep). Since small planktonic copepods are dominant in the plankton communities in many parts of the world's oceans and consequently are important members of pelagic food webs (Turner, 2004),a positive pCO_2 effect on this major zooplankton components could have a crucial impact on the transfer of energy to higher trophic levels thus affecting e.g. future fisheries (Sswat et al.;Moyano et al., 2009).

Copepod species that do not exhibit vertical migration behaviour are considered as evolutionarily less exposed to high-pCO_2 levels compared to other copepods, and typically more sensitive to OA (Fitzer et al., 2012;Lewis et al., 2013). Accordingly, we firstly expected cyclopoid (dominated by *Oithona* sp.) and harpacticoid copepods (dominated by *Microsetella* sp.) to show lower abundances under elevated pCO_2 conditions as neither species shows diel migrations (Maar et al., 2006). However, during this experiment, elevated pCO_2 did not cause a significant effect on Cyclopoida and Harpacticoida abundances, according to the GAMM analyses (Fig II-4B and C). The reason for the decay in Cyclopoida and Harpacticoida abundances is unclear, but a possible explanation could be the distribution of the copepods in the water column, closer to the sediment traps, as it was previously observed in other experiments (Bach et al. 2016a; Algueró-Muñiz et al. 2017). *Oithona* and *Microsetella* have been reported to concentrate on marine snow (Ohtsuka et al., 1993;Koski et al., 2005) and during the present experiment, the cumulative flux of particulate organic matter to the sediment traps increased after DW addition (Stange et al., submitted). This might have promoted a downward migration of the copepods –already from the beginning of the

experiment on *Microsetella*- to enhance their feeding on sinking material, preventing us to sample them in the net hauls. Our results do not confirm a close connection between copepod migration behaviour and OA sensitivity, but provide information about responses of these under-studied copepod taxa in a late-winter bloom.

4.4 pCO_2 effects on *Oncaea* sp. and *O. dioca* interactions in pre- and post-bloom conditions

Oncaea's feeding strategies are associated with surface materials, such as fine particles, bacteria, or the tegument fluid of gelatinous zooplankton (*Sagitta* spp., *Oikopleura* spp. and *Salpa* spp.) (Go et al., 1998). During this study, abundances of *Oncaea* sp. and *O. dioica* (juveniles) were inversely correlated, as previously observed at other study sites (Itoh et al., 2014). *Oncaea* sp. was positively affected by pCO_2, recording higher abundances under medium- and high-pCO_2 treatments from (approximately) the beginning of the experiment until the end of the phytoplankton bloom, on t35 (Fig II-4D). *O. dioica* analysis showed some similarities with other studies at elevated nutrient concentrations (Troedsson et al., 2013). We found a positive correlation between *O. dioica* abundances and NOx (p-value = 0.0463) and total microZP abundances (p-value = 0.0205) both in the oligotrophic and the upwelling phases. However, unlike Troedsson et al. (Troedsson et al., 2013), we did not detect a significant pCO_2 effect on *O. dioica* when studying the whole experimental period (Fig II-4F). After DW addition, we observed that *O. dioica* completely disappeared under high-pCO_2 while *Oncaea* abundances were higher than in the other two treatments, suggesting a top-down control of *Oncaea* sp. on *O. dioica* abundances. Hence, the fact that during the last sampling days *Oncaea* sp. abundances decayed in the high-pCO_2 treatment might reflect the scarcity of *O. dioica* as food resource. Medium- and high-pCO_2 treatments seemed to have caused higher *O. dioica* abundances before DW addition, although those did not render to be significant. Concerning *Oncaea* sp. females' condition (Figs II-6 and II-7), we observed smaller individuals, as well as a higher number of immature females and a lower number of egg-carrying mature females in the high-pCO_2 treatment. However, unlike the major sensitivities to OA previously described for early life stages of calanoid copepods (Pedersen et al. 2013; Algueró-Muñiz et al. 2017), we did not observe a stronger pCO_2 effect on copepodites than on adults of *Oncaea* sp. (Fig II-5). We conclude that the negative pCO_2 effect detected on *Oncaea* sp. females'

reproductive output might cause adverse effects in the long term in those tropical and subtropical communities dominated by this species (e.g. (Böttger-Schnack, 1994)), especially in those where oncaeid copepods are the main prey for larvae and juvenile fish (Itoh et al. 2014). The lack of published OA research on *Oncaea* sp (Poecilostomatoida) makes the analysis presented here of special relevance, and calls for multigenerational OA studies on this species.

4.5 Influence of OA on the transfer of energy within the plankton community

As discussed above, community effects and trophic interactions may determine sensitivities to OA (Rossoll et al., 2013), which in turn may have an effect on the efficiency of the food web (Calbet et al. 2014; Cripps et al. 2016; Algueró-Muñiz et al. 2017). The autotrophic community was expected to experience an increase in biomass (Gismervik et al., 2002) responding to the nutrient input created by the DW addition. However, under the same nutrient enrichment conditions, a significant effect of CO_2 on plankton succession was observed during this experiment (Taucher et al. 2017a), suggesting that phytoplankton boost was likely faster under high-pCO_2. This situation could in turn cause a CO_2-dependant reduction in trophic efficiency after DW addition, due to the limited capacity of micro- and mesozooplankton grazers to use the boosted phytoplankton production (Calbet et al., 2014). Accordingly, the A:H ratio (autotrophy/heterotrophy) was the highest after DW addition –or, more precisely, during the phytoplankton built-up in the high-pCO_2 treatment-. TTE decreased in all three pCO_2 treatments during the phytoplankton bloom (t25-t35), and lowest TTE was detected under high-pCO_2 conditions, likely because under these conditions microZP might not have had enough edible food to react at the beginning of the bloom, consequently affecting mesozooplankton production (Riebesell et al., in prep). These results are in the line with previous studies (Calbet et al., 2014;Cripps et al., 2016) which point at a more-autotrophic and less-efficient food web under more high pCO_2 conditions when the consumers mismatch the phytoplankton bloom (Edwards and Richardson, 2004;Calbet et al., 2014), as observed during this experiment until ~t40. The increase in calanoid copepods recruitment observed in both high- and medium-pCO_2 treatments towards the end of the experiment points at pCO_2-induced effects under nutrient-repleted conditions, which could travel up the food web

reaching secondary consumers, as previously observed in eutrophic systems (Algueró-Muñiz et al. 2017; Sswat et al. submitted). In case of the medium-pCO_2 treatment, an increased grazing pressure of copepods (Calanoida) on dinoflagellates could explain that TTE in medium-pCO_2 was lower than in the other two treatments after the phytoplankton bloom. Our results thus suggest that pCO_2 effect on plankton succession depend on the coupling of the phytoplankton bloom with the grazers, ultimately affecting the development of the plankton community and the efficiency of the system.

Based on this study, end-of-century pCO_2 levels are not expected to cause major effects on subtropical zooplankton communities during oligotrophic phases. However, in bloom and post-bloom conditions, elevated pCO_2 might promote higher zooplankton abundances by bottom-up effects of CO_2-enhanced primary production. Hence, pCO_2-fuelling effects would reach grazers and travel up throughout the food web, increasing the transfer of energy to copepods and higher trophic levels. This could be extremely relevant in oligotrophic environments with short bloom periods such as the Canary Islands, where zooplankton biomass has been shown to have direct implications on larval abundance in different fish species during late winter bloom (Moyano et al., 2009). Therefore, a positive effect of pCO_2 on zooplankton abundance after a bloom event might eventually benefit larval recruitment, and consequently have an effect on future fisheries.

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Supplementary information

S1 Table: Biomass conversion factors. Only common species (species that represent >0.5% total catch (i.e. > 3540 ind), t1-t55) were considered for mesozooplankton biomass estimation. Carbon content was estimated for *Doliolum* sp. and *Oncaea* sp.from last sampling day (t56) samples.

MESOZOOPLANKTON	Conversion factor ($\mu g\ C\ ind^{-1}$)	Reference
Clausocalanus spp./*Paracalanus* spp.	0.339	averaged for *Paracalanus* sp. after Uye (2014)
Copepoda nauplii	0.04	length-carbon relationship for *Oithona similis*, from Sabatini & Kiørboe (1994)
Doliolum sp.	2.18	this study
Foraminifera	0.75	average for *Elphidium* (Moodley 2000)
Microsetella sp.	0.268	averaged for *Microsetella norvegica* after Uye (2014)
Nannocalanus minor	0.339	based on *Clausocalanus* spp./*Paracalanus* spp.
Oikopleura dioica; juveniles	1.178	avergaed for juveniles ~500 µm trunk length after King (1980)
Oithona sp.	0.58	Kiørboe & Sabatini (1994)
Oncaea sp.	2.7	this study
MICROZOOPLANKTON	Conversion factor	
Ciliates	$0.76\ V^{0.819}$ pg C $cell^{-1}$	Menden-Deuer & Lessart (2000)
Dinoflagellates	0.19 pg C mL^{-1}	Putt & Stoecker, 1989
PHYTOPLANKTON	Conversion factor ($pg\ C\ cell^{-1}$)	
Dinoflagellates	$0.76\ V^{0.819}$	Menden-Deuer & Lessart (2000)
Diatoms	$0.288\ V^{0.811}$	Menden-Deuer & Lessart (2000)
Diverse	$0.216\ V^{0.939}$	Menden-Deuer & Lessart (2000)

CHAPTER III

Direct and indirect impact of near-future pCO_2 levels on zooplankton dynamics

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Abstract

Ocean acidification has direct physiological effects on organisms by, for example, dissolving the calcium carbonate structures of calcifying species. However, non-calcifiers may also be affected by changes in seawater chemistry. To disentangle the direct and indirect effects of ocean acidification on zooplankton growth, we carried out a study with two model organisms. We investigated the individual effect of short term exposure to (1) high and low seawater pCO_2 and (2) different phytoplankton qualities as a result of different CO_2 incubations on the growth of a heterotrophic dinoflagellate and a copepod species. It has been previously observed that higher CO_2 concentrations can decrease phytoplankton food quality in terms of carbon:nutrient ratios. We therefore expected both seawater pCO_2 (pH) and phytoplankton quality to result in a decrease of zooplankton growth. Although we expected lowest growth rates for all zooplankters under high seawater pCO_2 and low algal quality, we found that direct pH effects on consumers seem to be of lesser importance than the associated decrease in algal quality. The decrease of primary producers' quality under high pCO_2 conditions negatively affected zooplankton growth, which may lead to lower availability of food for the next trophic level and thus potentially affect the recruitment of higher trophic levels.

1. Introduction

Human industrial activities have increased atmospheric carbon dioxide (CO_2) concentrations which have now reached values of over 400 ppm on average (Tans and Keeling 2013), the highest level for millions of years (Royer 2006; Pagani et al. 2011). About 25% of the CO_2 enters the oceans which can act as carbon (C) sinks (Canadell et al. 2007). As a result, the carbonate chemistry of oceans has changed, especially in the upper 100 meters where ocean acidification has a major influence (Doney et al. 2009).

Ocean acidification negatively affects a number of organisms directly by, for example, dissolving the calcium carbonate structures of calcifying species (Orr et al. 2005). However, marine organisms, such as micro- and mesozooplankton, may be subjected to other adverse effects. Lower seawater pH resulting from increasing pCO_2 could directly affect the physiology of both phyto- and zooplankton by changing intracellular pH, membrane potentials, and enzyme activities (Nielsen et al. 2010). This acidification of body fluids is known as hypercapnia (Fabry et al. 2008). When CO_2 levels increase in seawater, dissolved CO_2 more readily diffuses across body surfaces and equilibrates in both intra- and extracellular spaces. As in seawater, CO_2 reacts with internal body fluids causing H^+ ions to increase and pH to decrease. Hypercapnia can cause the suppression of metabolic processes (Michaelidis et al. 2005; Pörtner 2008) and disrupt acid-base homeostasis (Miles et al. 2007), thus decreasing growth rate and reproductive success and increasing mortality of marine organisms (Yamada and Ikeda 1999). Smaller organisms are likely to be more affected by changes in seawater chemistry than larger ones as a result of the differences in volume to surface ratios and future conditions will increase variations in pH at the cell surface (Flynn et al. 2012). However, despite their ecological importance, only few studies have focused on the impact of ocean acidification on microzooplankton so far, and, to our knowledge, their sensitivity to hypercapnia has never been investigated. Microzooplankton are an essential component in planktonic ecosystems. Indeed, they often comprise the major predatory group in microbial food webs (Sherr and Sherr 2002), and microzooplankters form a trophic link between pico-, nano- and microplankton on the one hand and higher trophic levels, such as copepods, on the other hand (Sommer et al. 2005). Although there is indication that microzooplankton are sensitive to elevated pH (Hinga 2002; Pedersen and Hansen 2003a), to our knowledge no studies have directly tested the effects of CO_2-induced lowering of the pH on marine

microzooplankton. The few existing experimental studies were carried out using natural plankton communities and observed no effects of a high pCO_2/low pH on microzooplankton, independent on whether the systems were fixed pH, or whether pH was allowed to drift as it would in reality (Suffrian et al. 2008; Rose et al. 2009; Aberle et al. 2013). Ocean acidification does not only reduce the global base pH but also influences smaller-scale fluctuations. For example, ocean acidification may influence spatial and seasonal variations by modulating seawater alkalinisation during intense C-fixation associated with phytoplankton blooms (Flynn et al. 2012). Having said this, as the knowledge on effects of ocean acidification on microzooplankton is so limited we decided to focus on effects of fixed changes in pCO_2 only. However, it is generally difficult to observe physiological effects in natural community experiments whereas smaller and more controlled microcosm studies are more helpful to understand physiological responses such as microzooplankton sensitivity to hypercapnia. Further, most studies measuring copepod physiological performance at lower seawater pH observed that copepods are relatively tolerant to hypercapnia (Mayor et al. 2012; McConville et al. 2013). Yet, recent studies observed strong negative effects of near-future ocean acidification levels on copepods (Lewis et al. 2013; Thor and Dupont 2015), and others suggest that inappropriate experimental designs might have underestimated the actual direct effect of ocean acidification on copepods, and potentially microzooplankton (Cripps et al. 2014a; Cripps et al. 2014b). Since micro- and mesozooplankton play different roles in the pelagic ecosystem, it is important to determine whether those two groups are differently affected by high pCO_2.

Apart from direct acidification effects, the increasing C availability in the marine environment will likely change primary productivity and the quality of phytoplankton as food for higher trophic levels (Low-Décarie et al. 2014). As primary producers reflect the nutrient composition of their surrounding medium, they are expected to show higher C:nutrient ratios as CO_2 availability increases (Burkhardt et al. 1999; Urabe et al. 2003; van de Waal et al. 2010). Further, algae with high C:nutrient ratios are known to often be food of inferior quality for herbivorous consumers since there is a larger difference between resource chemical composition and consumer metabolic requirements (Sterner and Elser 2002). Recent results indicate negative indirect effects of ocean acidification on copepods caused by a decline in prey quality when grown under high pCO_2 (Rossoll et al. 2012; Schoo et al. 2013). In the light

of the predicted increase of pCO_2 and the observed trend of decreasing nitrogen (N) and phosphorus (P) inputs to coastal areas (Grizzetti et al. 2012), the potential for an excess of C and a concurrent nutrient limitation at the base of the food web is considerably heightened. Although evidence is increasing that the growth rate of zooplankters decreases with increasing CO_2 availability to the algae (Olson and Kawaguchi 2011; Rossoll et al. 2012; Schoo et al. 2013), it remains unclear whether direct or indirect effects on consumer fitness play the more prominent role. Indeed, only one study investigated the direct and indirect effects of acidification on zooplankton growth and reproduction (Rossoll et al. 2012). This study concluded that high CO_2 availability decreases copepods fitness mostly indirectly. Further, Melzner (2011) showed that the effects of high CO_2 may be less pronounced when there is enough food available, as the energy needed to deal with the unhealthy environment is available. If energy is the limiting compound in the study of Melzner (2011), at low food concentrations higher algal C:nutrient ratios might benefit herbivores since algae grown under high CO_2 conditions are more energy rich as they often contain more lipids (Rossoll et al. 2012). The generality of this result remains unclear, as trophic upgrading and differential algae sensitivity in terms of growth rate and stoichiometry to pCO_2 may compensate for low food quality. For instance, Isari et al. (2015a) recently observed that increased pCO_2 does not affect the stoichiometric quality of the phytoplankton species *Heterocapsa sp.* and, logically, did not alter performances of copepods feeding on that prey. Further, Klein Breteler et al. (1999) showed that inadequate algal food could be biochemically upgraded by protozoans to high quality food for copepods. Hence, protozoan herbivores might dampen the negative effect of acidification on algal food quality through trophic upgrading. In fact, Caron & Hutchins (Caron and Hutchins 2012) identified lack of data on the effects of ocean acidification on microzooplankton as one of the major knowledge gaps.

To disentangle the direct and indirect effects of ocean acidification on both microzooplankton and mesozooplankton, we carried out a study with model organisms. We investigated the individual effect of short term exposure to (1) high and low seawater pCO_2 and (2) different qualities of the alga *Rhodomonas salina* on the growth and development of two model zooplankton species *Oxyrrhis marina* (Montagnes et al. 2010) and *Acartia tonsa* (Mauchline 1998). The different algal qualities were obtained by growing *R. salina* in high and low seawater pCO_2 relative to current scenarios. We hypothesize that a significant interaction of

seawater pCO_2 and phytoplankton quality should affect zooplankton growth and that lowest growth rates for both micro- and mesozooplankters should be observed under high pCO_2 seawater and low algal quality.

2. Material & Methods

To test whether planktonic herbivores growth is affected by seawater pCO_2 or by algal quality differences caused by seawater pCO_2 (direct versus indirect effect), we conducted a laboratory experiment with nauplius and copepodite stages of the copepod *A. tonsa* as well as with the heterotrophic dinoflagellate *O. marina*. The zooplankton species were cultured under high and low pCO_2 and were fed with two algal qualities in full factorial design, i.e. four treatments. The different algal qualities were obtained by growing the model organism *R. salina* in high and low seawater pCO_2 relative to current scenarios. Using model organisms such as *O. marina* and *R.* salina entails limitations regarding the extent to which experimental results can be interpreted. Nevertheless, due to its high growth rate and to the reproducibility of nutrient treatments, *R. salina* is a useful model organism when studying the importance of phytoplankton food quality for zooplankton. Further, Davidson et al. (2010) made a critical assessment of the advantages and disadvantages of using *O. marina* as a model organism. Their study supports the use of this dinoflagellate in experimental studies since its feeding mode and predator:prey size ratio are comparable to most protozoa. Both *O. marina* and *R. salina* are planktonic, not benthic, and in this sense, they are appropriate model organisms for planktonic processes.

Phytoplankton

R. salina (Wislouch) Hill et Wetherbee was kept in F/2 medium prepared with 0.2µm filtered seawater. *R. salina* was grown in continuous chemostat cultures maintained at steady state. The phytoplankton cultures were constantly aerated with a mixture of air stripped of CO_2 by soda lime and pure CO_2 adjusted to 200 and 800 µatm (Rho 200 and Rho 800) to represent pre-industrial and predicted future scenarios. The pre-defined pCO_2 level was achieved following Schoo et al. (2013). A sensor (HTK Hamburg) continuously monitored the pCO_2 of

the gas mixture distributed to the algal cultures and automatically adjusted the CO_2 content and flow rate. Preliminary experiments showed that the pCO_2 of the growth medium was in equilibrium with the target level of the gas pCO_2 within 1 h of aeration. The algal chemostat cultures (5 L) were stirred continuously and kept at 18°C under a 16h:8h light:dark regime (185µmol m^{-2} s^{-1}). Subsamples from the surplus culture collected in an overflow container were taken daily to measure the cell density of the cultures with a CASY cell counter (SCHÄRFE SYSTEMS, Reutlingen, Germany) as well as the algal stoichiometry (see procedure below).

To feed the zooplankton populations, two new *R. salina* batch cultures were created daily at concentrations of 0.5 * 10^{-6} cells L^{-1} to ensure that, within each treatment, zooplankters were feeding on the same algal quality for the duration of the experiment. We needed to prepare batch cultures since the volume of algae needed to feed the zooplankton populations was too important to be taken from the chemostats. The phytoplankton cultures were constantly aerated with air at pCO_2 of 200 and 800 µatm (Rho 200 and Rho 800). It is important to note that this study was conducted using a controlled system with fixed pH which might induce different responses than in the field where pH drifts with phytoplankton growth (Flynn et al. 2015). The algae were cultivated in 1 L batch cultures in F/2 medium at 18°C under a 16h:8h light:dark regime (185µmol m^{-2} s^{-1}). After three days of growth, an aliquot of each *R. salina* culture was filtered onto pre-combusted Whatman GF/F filters. The particulate C and N content of *R. salina* was measured with a Vario Micro Cube/CN-analyser (Elementar). Particulate P was analysed as orthophosphate after acidic oxidative hydrolysis with 5% H_2SO_4 (Grasshoff et al. 1999).

Microzooplankton

O. marina Dujardin was obtained from the Göttingen culture collection (Strain B21.89) and fed *R. salina* at 18°C under a dim continuous light regime (50µmol m^{-2} s^{-1}). Prior to the experiment, the *O. marina* culture was starved for 1 week in order to eradicate any effects of preculture conditions. This culture was then split into 28 separate cultures (four treatments, seven replicates) which were all diluted to a start concentration of 20,000 cells mL^{-1} with CO_2 preconditioned artificial, sterile and nutrient-free seawater (Aqua Marin) at a salinity of 32. Cell concentrations of the cultures were determined using a CASY particle counter (SCHÄRFE

SYSTEMS, Reutlingen, Germany). The *O. marina* cultures were gently aerated with a mixture of air and pure CO_2 adjusted to 200 (Water200) and 800 µatm (Water800). As for phytoplankton cultures, a sensor continuously monitored the pCO_2 of the gas mixture distributed to the algal cultures and automatically adjusted the CO_2 content and flow rate. Pre-experiments indicated that gentle bubbling does not affect *O. marina* growth rate. The cultures had pH of 8.3 (±0.06) and 7.8 (±0.05) when aerated with 200 and 800 pCO_2, respectively. The total alkalinity of the CO_2 preconditioned artificial seawater was 3300 µmol L^{-1} for the 200 µatm and 3197 µmol L^{-1} for the 800 µatm pCO_2 treatment. The pH was measured with a ProLab 3000 pH meter with an IoLine pH combination electrode with temperature sensor (type IL-pHT-A170MFDIN-N). TA was estimated from open-cell duplicate potentiometric titration and calculation with modified Gran plots (Bradshaw et al. 1981), using a TitroLine alpha plus titrator with an IoLine pH combination electrode with temperature sensor (type IL-pHT-A120MF-DIN-N). The carbonate system was calculated from TA, pH, temperature and salinity using CO2Sys (Lewis et al. 1998), the pCO_2 values obtained were 292 and 911 ppm for the 200 and 800 treatments, respectively. To calculate the carbonate system, we used the equilibrium constants of Mehrbach et al. (1973) refitted by Dickson and Millero (1987). Each of the two pCO_2 culture sets (Water200 and Water800) was fed *ad libitum* with 35 algal cells per *O. marina* daily during four days with either Rho 200 or Rho 800 (Rho 200-Water 200, Rho 200-Water 800, Rho 800-Water 200, Rho 800-Water 800). The quantity of food given daily was sufficient to prevent food quantity limitation and was adjusted at the last feeding day to minimize the amount of remaining algal cells after 24h. After four days of growth, the cell concentration of each *O. marina* culture was determined by CASY counting and the relative growth rate of each culture was calculated.

Mesozooplankton

Eggs of the calanoid copepod *A. tonsa* were produced in 200-liter cylindrical tanks, where the animals were cultivated at 18°C at a 16:8 light:dark cycle. Copepods were fed with *R. salina*. Eggs were siphoned from the bottom of the tanks daily and stored in seawater at 4°C for later use. The stored eggs were incubated in fresh seawater at 18°C for hatching. Since hatching peaks between 24h and 36h of incubation, we collected the nauplii hatched during this period

to minimize age differences between individuals. Nauplii were placed at 18°C under a dim 16:8 light:dark regime in the same four treatments above-described for microzooplankton and fed daily for 12 days. The cultures were put in 1 L glass containers at densities of 1,000 individuals L^{-1} in seven replicates per treatment. Copepods were fed *ad libitum* with 20,000 algal cells per copepod and all experimental animals were washed daily over a sieve (75 µm mesh size) to separate them from any algae before being introduced to fresh CO_2 preconditioned artificial seawater prior to feeding in order to minimise changes in the nutrient composition of the algae by waste products of the animals. The developmental rate of the juvenile copepods was determined after 12 days. The developmental stages of the copepods were determined for at least 100 individual animals from subsamples at the end of the experiment. For the calculation of the developmental rates, all naupliar larvae were assigned to stage 6, the last naupliar stage before transition to the copepodite stages. The number of individuals per stage was divided by the number of days of growth, and the developmental rate per treatment was then calculated by dividing this sum by the number of individuals counted. We also tested the copepods reaction to the treatments by measuring the respiration rates of stage 4 nauplii and stage 3 copepodites. Copepods respiration rates were measured following the procedure described by Schoo et al. (2013). Respiration rates were determined with a microsensor oxygen metre (PreSens Precision Sensing, Germany) equipped with oxygen microoptodes. Approximately 100 nauplii and 75 copepodites were sampled from the incubation containers at day 5 and 10 and were washed over a sieve (75 µm mesh size) to separate them from any algae before being introduced into the 5 mL incubation vessel. Oxygen air saturation values were below 80 % at the end of the 1h measurements. Bacterial respiration rates were measured as a control treatment at the same time and the measured bacterial respiration rates were deduced from the total respiration rates of the copepod measurements. The animals were collected and counted after the incubation to determine the precise number of animals in each vessel, permitting an accurate calculation of respiration rates per individual animal. Respiration rates were calculated by linear regression of oxygen concentration over time. Technical issues unfortunately prevented us from measuring the respiration for *O. marina*. It is not possible to separate *O. marina* from *R. salina* due to small size differences between the two species and pre-experiments showed that using a control with algae only generates too large standard deviations.

3. Results

The population density and the stoichiometry of the algae grown in the continuous chemostat cultures were affected by the exposure to the different *p*CO2 during growth (Fig III-1). The phytoplankton cultures had significantly higher cell densities when grown under elevated *p*CO2 (Fig III-1A, Repeated Measures ANOVA, $F_{2,19}$ = 44.46, P<0.01). Both C:N (Fig III-1B, Repeated Measures ANOVA, $F_{2,19}$ = 44.46, P<0.01) and C:P ratios (Fig III-1C, Repeated Measures ANOVA, $F_{2,18}$ = 159.57, P<0.01) were significantly higher in *R. salina* cultures reared under elevated pCO_2.

We cultured zooplankton in low and high pCO_2 seawater and fed them algae grown in batch cultures under low and high pCO_2. The CO_2 treatments significantly affected algal C content; Rho 800 was 30% richer in C than Rho 200 (Table III-1, t-test p<0.05). This difference in C content resulted in lower C:N (10.1 ± 3.1) and C:P ratios in Rho 200 (294 ± 24) than in Rho 800 (14.7 ± 0.9 and 396 ± 31; t-test p<0.05). Further, the CO_2 treatments did not affect the N and P content and the N:P ratio of *R. salina*.

Table III-1: Mean carbon, nitrogen, phosphorus cell content (pg $cell^{-1}$) and C:N:P of *R. salina* used to feed the zooplankton cultures. Numbers in brackets are standard deviations of five replicates and stars indicate significant differences (n = 5; FG = 8; P<0.05).

	Rho 200	Rho 800
C (pg $cell^{-1}$)	57.2 (1.1)*	79.5 (0.6)*
N (pg $cell^{-1}$)	6.2 (1.1)	6.2 (0.4)
P (pg $cell^{-1}$)	0.57 (0.01)	0.53 (0.03)
CN (molar)	10.1 (3.1)*	14.7 (0.9)*
CP (molar)	294 (24)*	396 (31)*
NP (molar)	22.6 (3.4)	26.3 (3.8)
Growth rate (d^{-1})	0.42 (0.04)	0.43 (0.03)
ESD (µm)	9.46 (0.08)	9.81 (0.12)

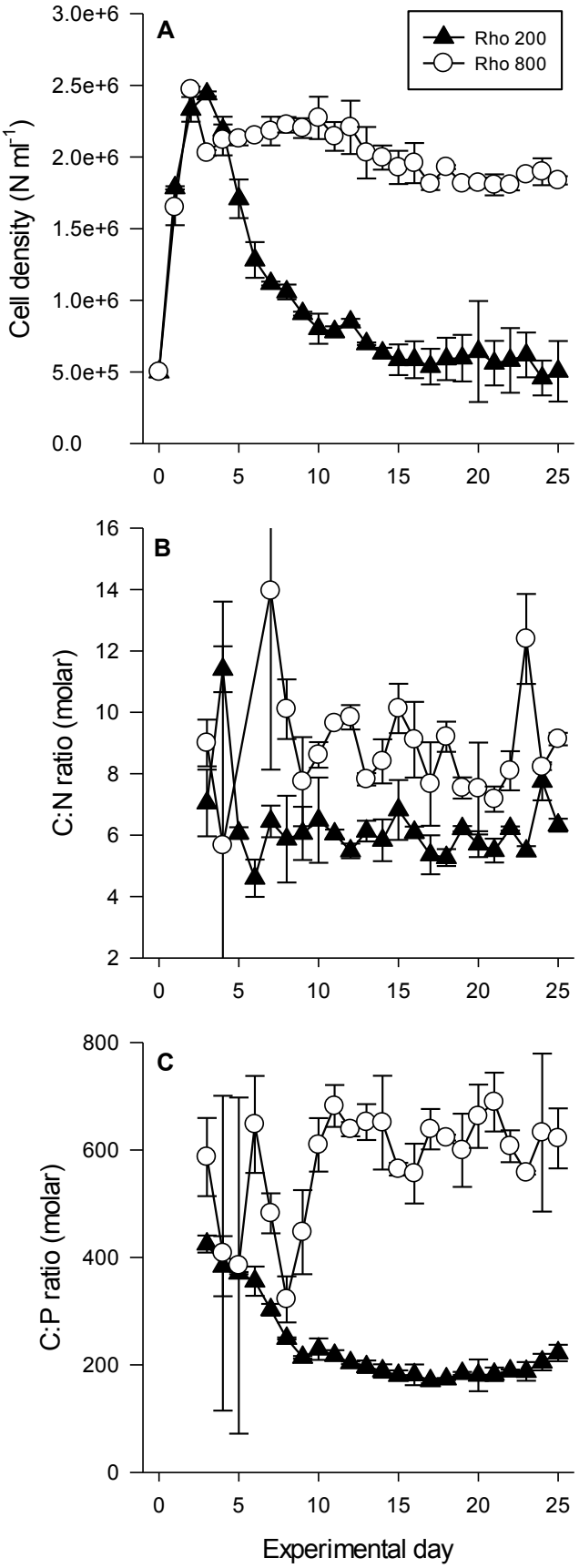

Fig III-1: *R. salina* (A) cell densities, (B) C:N ratios, and (C) C:P ratios grown in chemostats under different pCO_2. Data presented are means and standard deviations of three replicates.

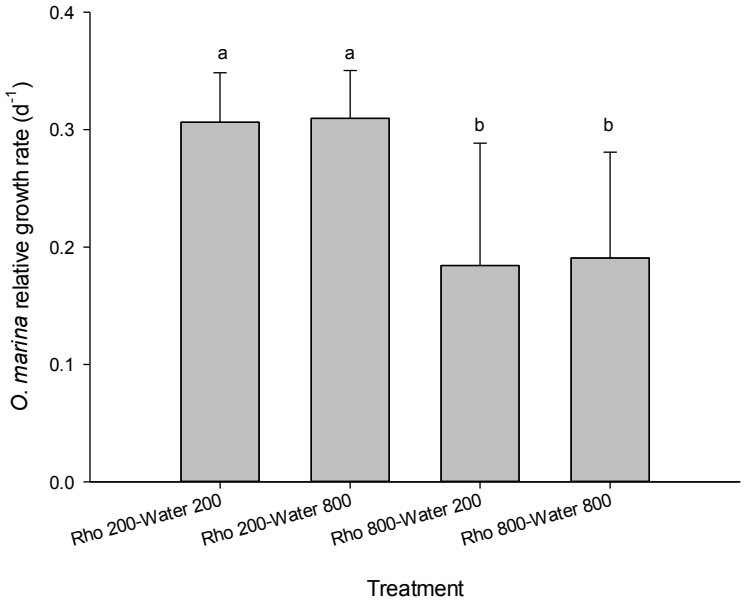

Fig III-2: Growth rates of *O. marina* reared under different pCO_2 and fed with different pCO_2 *R. salina*. Data presented are means and standard deviations of seven replicates. Statistically significant differences ($p<0.05$) are indicated by letters.

Although one may argue that the change in algal C:N:P reflects a short-term response caused by the small duration of adaptation, our chemostat culture indicates that such changes persist over generations (Schoo et al. 2013).We observed that the growth rate of *O. marina* was significantly affected by the food quality treatment (Fig III-2, two-way ANOVA, $p<0.05$), while seawater pCO_2 did not alter the dinoflagellate growth. *O. marina* growth rate was 40% lower when feeding on the C-rich Rho 800 (0.18 d^{-1}) than when feeding on Rho 200 (0.3 d^{-1}). Further, although we expected to observe the lowest growth rate under high pCO_2 seawater and low algal quality, the ANOVA interaction effects between seawater pCO_2 and algal quality did not significantly affect the growth of *O. marina* (two-way ANOVA $p>0.05$). Similarly, only algal quality had an effect on the development of *A. tonsa* (Fig III-3). While seawater pCO_2 did not affect *A. tonsa* development, Copepod populations feeding on C-rich Rho 800 contained a lower percentage of the older C6 and C5 copepodite stages at the end of the 12 days growth experiment than those feeding on Rho 200 (Fig III-3A). Consequently, *A. tonsa* developmental rates were influenced by algal quality and we observed a significant development decrease in the Rho 800 treatment compared to the Rho 200 treatment (Fig III-3B, two-way ANOVA, $p<0.05$); while seawater pCO_2 had no significant effect. The different food qualities also led to differences in copepod respiration rates. Copepods feeding on C-rich Rho 800 had significantly higher respiration rates than those feeding on Rho 200 (Fig III-4, two-way ANOVA, Tukey's honest significant difference posthoc test, $p<0.01$); while no effect of seawater pCO_2 could be identified. Further, although we expected the combination of high pCO_2 seawater

and low algal quality to have a strong effect, the ANOVA interaction effects between seawater pCO_2 and algal quality did not significantly affect the development and respiration of *A. tonsa* (two-way ANOVA $p>0.05$).

4. Discussion

Although it has previously been suggested that smaller organisms should be more affected by ocean acidification (Flynn et al. 2012), we found no direct effect of seawater pCO_2 on the dinoflagellate and copepod species we studied. This indicates that zooplankton might already be resistant to hypercapnia. Due to environmental variability (*e.g.*, upwelling, rock pools), diapause at depth, many zooplankton (including larval stages) already face pH levels much lower than those predicted for surface waters in the coming century (Olson and Kawaguchi 2011). Thus, predicted changes in surface seawater pH may be small relative to the range of pH zooplankton experience during their lifespan. These organisms could already be well adapted to seawater pH variations and potential effects of hypercapnia.

Our study, however, shows that the primary producer used in this study increased its cellular carbon content when cultured under elevated pCO_2. Both higher C fixation and increased growth rate under high pCO_2 could result in increased C:nutrient ratios. Culturing *R. salina* under different pCO_2 at identical dilution rates (i.e. growth rates) in chemostats yielded different C:nutrient ratios (Fig III-1, see also Schoo et al. 2013). This indicates that elemental stoichiometric differences are caused by higher C fixation rather than by higher growth rates under high pCO_2. This change in algal biochemical composition, and therefore quality, decreased the growth of the dinoflagellate *O. marina* as well as the development of the copepod *A. tonsa*. Thus, not only copepodites (as shown by Schoo et al. 2013) but also nauplii and microzooplankton react with decreasing growth with increasing CO_2 availability to the algae. Altogether, the growth rate and development of microzooplankton and mesozooplankton decrease at higher pCO_2, coupled with the suppression of reproductive scope identified by other studies (Cripps et al. 2014a; Cripps et al. 2014b) have clear potential to damage population growth dynamics.

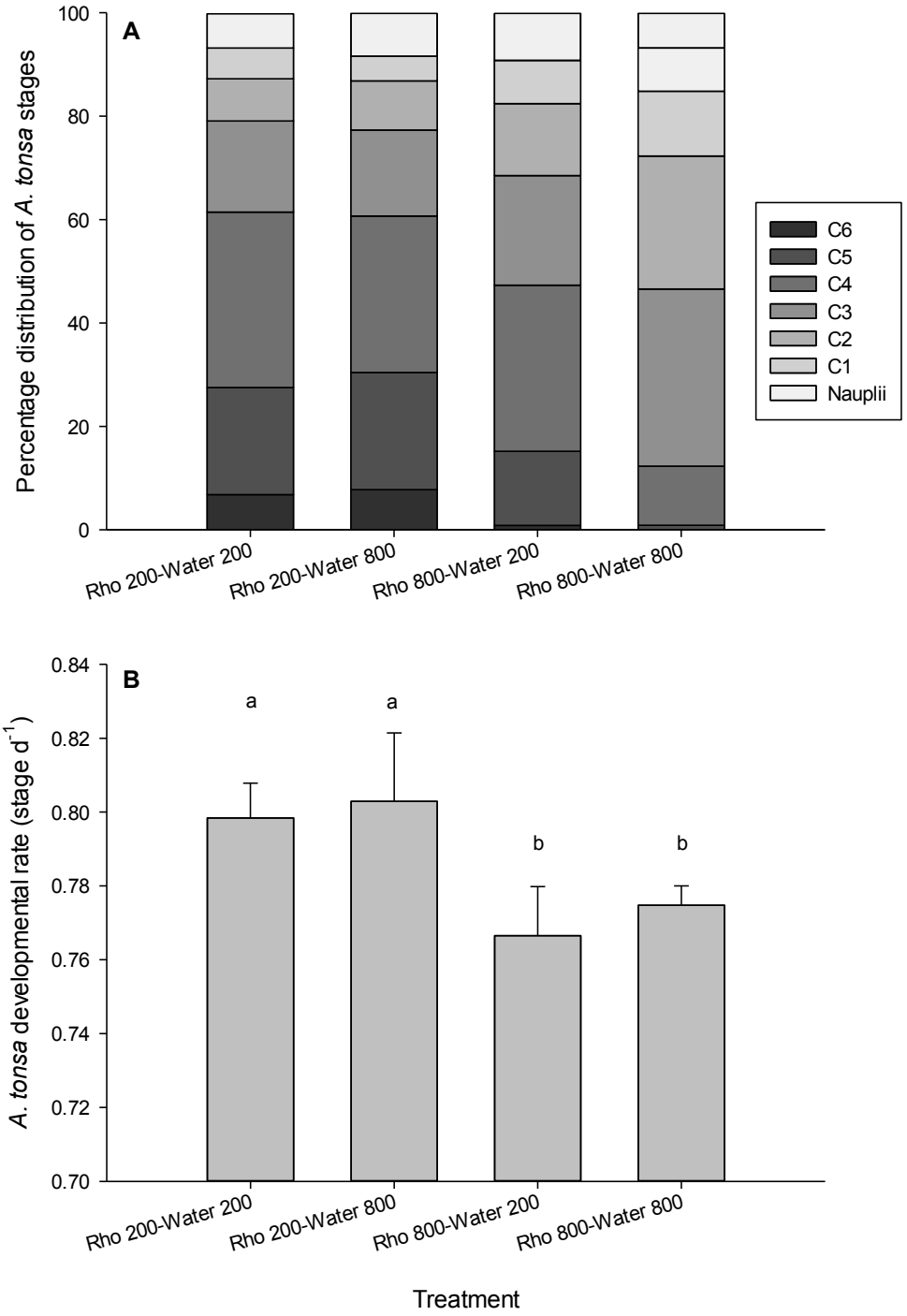

Fig III-3: (A) Percentage distribution and (B) developmental rates of *A. tonsa* development stages reared under different pCO_2 and fed with different pCO_2 *R. salina*. Data presented are means and standard deviations of seven replicates. Statistically significant differences ($p<0.05$) are indicated by letters.

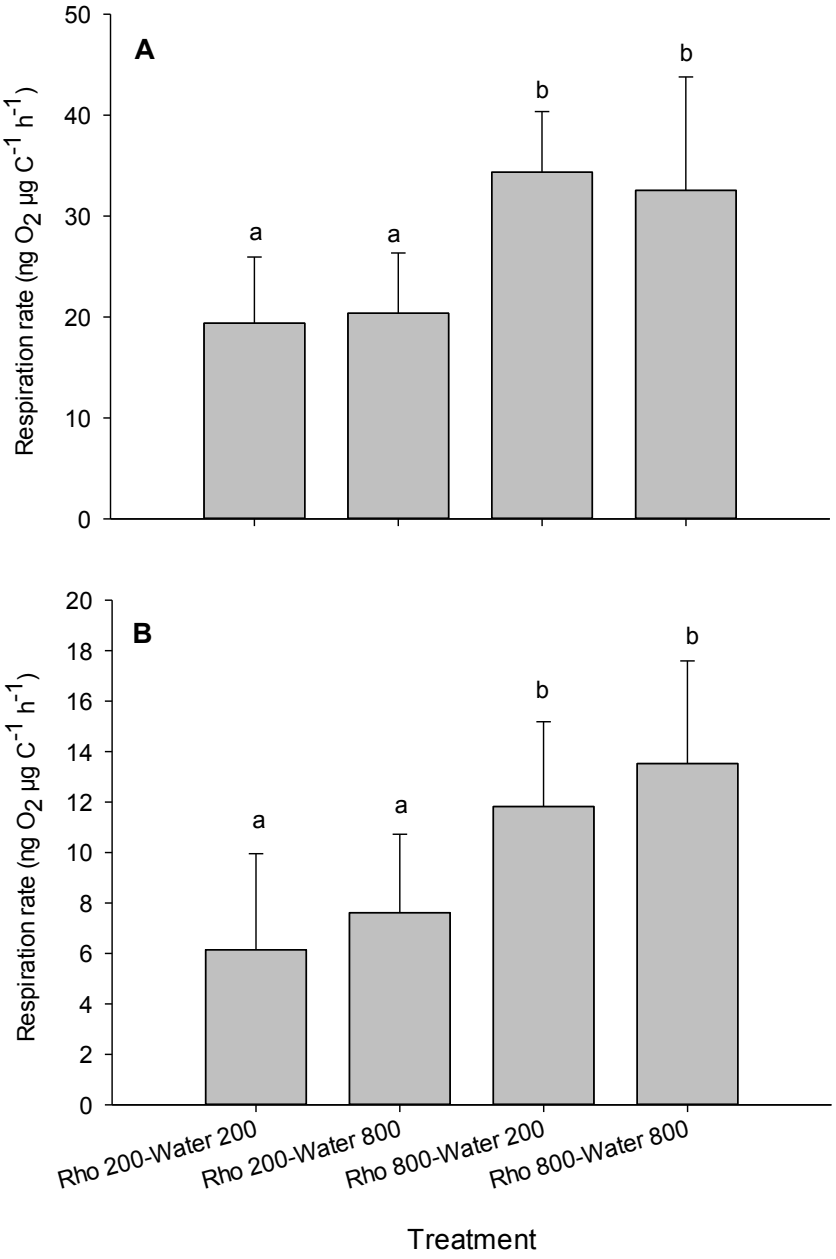

Fig III-4: Respiration rates of (A) *A. tonsa* nauplii and (B) copepodites reared under different pCO_2 and fed with different pCO_2 *R. salina*. Data presented are means and standard deviations of seven replicates. Statistically significant differences ($p<0.01$) are indicated by letters.

To our knowledge, none of the previous studies investigated the direct and indirect effects of near-future pCO_2 levels on zooplankton. Only Rossoll et al. (2012) conducted a full factorial experiment testing direct and indirect high pCO_2 effects on copepods. However, their work suffers from the fact that copepods did not contain any long chain fatty acids in acid treatments although they were provided by the algae (at decreased amounts). This points to an unexplained lack of feeding rather than an effect of food quality. Our results therefore bring important new knowledge on the impact of ocean acidification on copepods. Further, our study contradicts the conclusions of the few existing experimental studies on microzooplankton which found no effects of increased pCO_2 (Suffrian et al. 2008; Rose et al. 2009; Aberle et al. 2013). However, the major focus of those studies was on the direct effects of increased pCO_2, which, as we identified here, does not impact microzooplankton. Although we expected that the direct effect of high CO_2 would be most pronounced when the grazers were feeding on low algal quality, the interaction between these two treatments did not alter zooplankton performances. This could be the result of an elevated energy expenditure enabled by higher algal energy content and should result in higher grazers' respiration rates. However, nauplii and copepodites respiration was only increased by low algal quality. As previously described by Schoo et al. (2013), we suggest that increased respiration rates represented a physiological response to excrete the excess C obtained from prey grown in high pCO_2 conditions, rather than a stress response to deal with low pH.

In this study, we found that direct pCO_2 effects on consumers seem to be of lesser importance than the associated decrease in algal quality. Several studies have investigated the direct effects of ocean acidification on zooplankton. While elevated pCO_2 does not seem to affect adult copepods, hatching rates are negatively affected by very high pCO_2 (Kurihara et al. 2004; Cripps et al. 2014b). Further, the decrease of primary producers' quality under high pCO_2 conditions negatively affects zooplankton production and growth. However, the generality of this result remains uncertain, as community level dampening, such as species richness and complex trophic interactions, may compensate for low food quality (Rossoll et al. 2013). Indeed, the CO_2 effect in the one alga – one copepod species food chain in the study by Rossoll et al. (2012) vanished when the same zooplankton species fed on a semi-natural food mixture in mesocosms (Rossoll et al. 2013). Nevertheless, lower growth rates of zooplankton, as shown in this study, may lead to lower availability of food for the next trophic level and thus

potentially affect the recruitment of higher trophic levels. Furthermore, quality effects have also been shown to travel up the food chains (Malzahn et al. 2007), and decreased algal quality may affect higher trophic levels as well.

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CHAPTER IV

Withstanding multiple stressors: ephyrae of the moon jellyfish (*Aurelia aurita*, Scyphozoa) in a high-temperature, high-CO_2 and low-oxygen environment

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Abstract

Global change is affecting marine ecosystems through a combination of different stressors such as warming, ocean acidification and oxygen depletion. Very little is known about the interactions among these factors, especially with respect to gelatinous zooplankton. Therefore, in this study we investigated the direct effects of pH, temperature and oxygen availability on the moon jellyfish *Aurelia aurita,* concentrating on the ephyral life-stage. Starved one-day-old ephyrae were exposed to a range of pCO_2 (400 to 4000 ppm) and three different dissolved oxygen levels (from saturated to hypoxic conditions), in two different temperatures (5 °C and 15 °C) for seven days. Carbon content and swimming activity were analysed at the end of the incubation period, and mortality noted. General linearized models were fitted through the data, with the best fitting models including two- and three-way interactions between pCO_2, temperature and oxygen concentration. The combined effect of the stressors was small but significant, with the clearest negative effect on growth caused by the combination of all three stressors present (high temperature, high CO_2, low oxygen). We conclude that *A. aurita* ephyrae are robust, and that they are not likely to suffer from these environmental stressors in a near future.

1. Introduction

Human-driven climate change, and the associated changes in abiotic parameters, is challenging species and ecosystems worldwide. For instance, anthropogenic carbon dioxide (CO_2) emissions have modified the carbonate chemistry of the oceans causing ocean acidification (OA) concurrent with the rise in temperature, causing ongoing biological change in marine ecosystems (Perry et al. 2005; Rosenzweig et al. 2008). Ocean surface waters have experienced an increase of ~0.11 °C per decade during the last 40 years and are predicted to become warmer during the 21st century, increasing the temperature in the upper 100 m by 0.6–2.0 °C by 2100 (IPCC 2013). Additionally, a doubling or tripling in carbon dioxide concentrations is projected by 2100 (IPCC 2013). These increases in CO_2 affect biota not only directly by decreases in pH, but also indirectly via trophic pathways (Boersma et al. 2008; Malzahn et al. 2010; Schoo et al. 2013). In marine ecosystems, CO_2 and O_2 are stoichiometrically linked through respiration processes. Moreover, acidification and low oxygen availability are closely related in oxygen minimum zones (OMZ) (Brewer and Peltzer 2009; Paulmier et al. 2011; Melzner et al. 2013; Gobler et al. 2014). Especially in coastal areas, cultural eutrophication has led to an increase in hypoxia, thus linking acidification with eutrophication and the consumption of dissolved oxygen in bottom waters (Diaz and Rosenberg 2008; Rabalais et al. 2010; Wallace et al. 2014). Therefore, the loss of dissolved oxygen (DO) in the world's ocean - or "deoxygenation" - is another foreseeable change considering that O_2 is less soluble at warmer temperatures, and that increased stratification is predicted (Sarmiento et al. 1998; Bopp et al. 2002; Keeling and Garcia 2002; Keeling et al. 2010). End-of-century scenarios predict the deoxygenation trend to continue (IPCC 2013). As environmental drivers do not appear alone but act simultaneously, additively, or sometimes synergistically (Pörtner et al. 2005; Kirby et al. 2009; Bijma et al. 2013; Dupont and Pörtner 2013), it is generally not possible to extrapolate results from studies on single stressors to predict the impact of multiple stressors. Unfortunately, despite a large body of literature on the effects of individual stressors, only a handful of studies have considered ecophysiological responses to multiple environmental stressors. Further, most of those studies have focused on calcifying organisms (e. g. Melzner et al. 2013; Jansson et al. 2015; Queirós et al. 2015; Steckbauer et al. 2015), while non-calcifiers have remained understudied (but see: Kurihara 2008; Nguyen et al. 2012; Davis et al. 2013). Full factorial experimental designs are needed to

study the biotic changes associated with concurrently operating stressors such as ocean warming, acidification and hypoxia. Indeed, only approaches testing realistic scenarios in their entirety will allow a robust evaluation of future climate change effects on coastal and oceanic ecosystems (Riebesell and Gattuso 2015).

During recent years, several studies have linked climate variation and global gelatinous zooplankton blooms (Lynam et al. 2004; Purcell 2005; Purcell 2012), because of the purported tolerance of jellyfish (scyphomedusae, hydromedusae, siphonophores and ctenophores) to human-driven ecosystem changes (reviewed in Purcell et al. (2007)). Based on long-term datasets, some authors have claimed that there have been increases in the occurrence of regional blooms of some jellyfish, especially in overexploited areas (e. g. Brodeur et al. 2002; Lynam et al. 2006; Kogovšek et al. 2010). However, other studies have come to different conclusions, questioning the direct link between increases in jellyfish populations and anthropogenic change (e. g. Lynam et al. 2004; Condon et al. 2012; Gibbons and Richardson 2013). As most of the evidence is based on inference from field data, this calls for further experimental studies on the reaction of jellyfish to global change. Studies on scyphozoan (Lesniowski et al. 2015) and cubozoan polyps (Klein et al. 2014) have shown that polyps may thrive under future scenarios, however jellyfish occurrences also depend on polyps asexual reproduction. In scyphozoans, the size of the medusa population largely depends on the recruitment, reproduction, and survival of the early life-stages (Fu et al. 2014). Most likely, the sexually reproducing stage (medusa) is not the bottleneck for population development, but rather the preceding stages. Therefore, in order to predict jellyfish blooms, all life stages must be carefully considered when studying the effects of environmental changes.

The moon jellyfish (*Aurelia aurita,* Linnaeus 1758) is one of the best studied scyphozoans. Worldwide distributed from 70º N to 40º S, *A. aurita* is common in coastal areas within a wide range of environmental conditions, including polluted eutrophic systems (Lucas 2001 and the references therein). Recent studies report high tolerance of *Aurelia* sp. to ocean acidification, and no effect of lower pH on the number of statoliths (calcium sulphate hemihydrate crystals located in statocysts). Decreased pH, however, reduced the size of the statoliths (Winans and Purcell 2010), which could potentially affect orientation and swimming activities of the free-swimming stages (ephyrae and medusae). Tolerance and even positive effects of increasing temperatures were described in different life stages of *Aurelia* sp. (planula larva, polyp,

ephyra, and medusa) through higher metabolic rates, and sexual and asexual reproduction rates (Ishii and Takagi 2003; Holst 2012). Moreover, even though recent results indicate that low O_2 concentrations limit *A. aurita* medusa vertical distribution (Suzuki et al. 2016), at the same time they promote settlement of planulae (Ishii et al. 2008), favour polyps' asexual reproduction (Ishii et al. 2008), and reduce predation pressure during polyp development (Ishii and Katsukoshi 2010; Miller and Graham 2012).

In contrast, the knowledge on the ephyral stage of *Aurelia* sp. is still limited. Survivorship of *Aurelia* sp. ephyrae is low; less than 1 % survive to reach adulthood, but the causes of this high mortality remain elusive (Ishii et al. 2004). According to Fu and colleagues (2014) ephyra cumulative mortality in the field can reach ~95 % by age 4.6 days and increase further to ~99 % by the young medusa stage (20 to 28 day old). We are aware of only a few studies dealing with the effects of temperature on development and survival of ephyrae demonstrating that low temperatures lead to decreased feeding rates (e.g. Widmer 2005; Wang and Li 2015). Previous studies on *Aurelia labiata* indicate that the number of statoliths in ephyrae released at 7.2 pH-treatments did not differ but had significantly smaller volumes compared to higher pH-levels (7.5 and 7.9) (Winans and Purcell 2010). This could potentially affect orientation and swimming activities of the free-swimming stages (ephyrae and medusae). However, acids (HCl) and bases (NaOH) were added to the experimental treatments in this study on *A. labiata* which may not sufficiently reflect the seawater carbonate chemistry changes associated with ocean acidification (Gattuso and Lavigne 2009; Klein et al. 2014). The effects of hypoxia on ephyrae are unknown but also may reduce swimming activity and increase mortality due to negative effects on metabolic processes. Nonetheless, the potential interactive effects of these stressors could produce different, potentially more severe effects and thus provide a more realistic understanding of this species in a changing ocean context. Thus, there is a great need to fill this gap in our knowledge, especially investigating these stressors in concert.

In order to assess the tolerance of ephyrae of *A. aurita* to multiple stressors, we studied the direct responses to hypoxia, warming and ocean acidification using a full-factorial design. Previous studies have demonstrated that *Aurelia* spp. are tolerant to a wide range of abiotic environmental factors (Lucas 2001). Since this species is relatively resilient to metabolic stress (Cargo and King 1990; Cawood 2012), we hypothesize that if *A. aurita* ephyrae are affected by abiotic change, it will probably be because of the combined effects of multiple stressors.

2. Material & Methods

Animal collection and polyp culture of *Aurelia aurita*

During spring of 2014 adult female *Aurelia aurita* with oral arm brood pouches containing planula larvae were collected around the island of Helgoland, North Sea. The collected medusae were stored at 18 °C in the dark in 10 L plastic aquaria filled with filtered (1 µm) North Sea water (FSW). After 12 h, planula larvae released from these medusae were collected and transferred to 5 L plastic aquaria filled with FSW. Plastic petri dishes (~60 per 5 L aquarium, 35 mm diameter) were placed on the water surface to allow settlement of the planula larvae (Holst and Jarms 2007). Early developmental stages of the brine shrimp (*Artemia franciscana*) were used to feed the dense cover of small polyps which metamorphosed from the settled planulae on the underside of the floating substrates within two days. During the first three weeks after settlement, young polyps (about 20 per settling plate) were fed with mashed freshly hatched nauplii. Once the polyps reached the eight-tentacle stage they were fed with living *Artemia* nauplii. Two months after settlement, polyps were large enough to capture larger prey. From that date polyps were fed once per week with a mixture of different stages of *A. franciscana,* which was collected 24-30 h after hatching. In the feeding process, the food was added to every culture container, and the polyps were allowed to feed for four hours. Afterwards, the polyps were transferred to new containers with fresh FSW at 15 °C.

Ephyrae production

Polyps were reared in 12 aquaria at 15 °C in temperature-controlled rooms. In December of 2014, when most polyps were grown (about 3 mm in height), strobilation was induced by a temperature decrease from 15 to 10 °C (Holst 2012) in two daily steps of 2.5 °C. Strobilation started after 4-5 weeks at 10 ℃ (~90 % of the aquaria contained strobilae) and feeding was stopped in all the aquaria. To collect the ephyrae from the bottom of the culture containers one third of the water volume was siphoned with a glass pipette, and carefully filled up with fresh FSW at 10 ℃ to avoid disturbances of the strobilation process. The ephyrae harvested in the first 12 days of strobilation were not used for the experiment in order to homogenize the starvation regime and to obtain ephyrae in a similar condition. To obtain ephyrae in

approximately identical developmental stages, the totality of the free-swimming individuals was collected every day. Only ephyrae that were healthy, well-shaped, similar sized (3.75 ± 0.45 mm between opposite rhopalia) and 1-day-old were selected for the experiment, excluding all animals with a number of marginal lappets different to 8, or any other kind of visible malformation. Differences in ephyra size were impossible to avoid, but differently-sized animals were equally distributed among the treatments in sets of 5 ephyrae per experimental unit. Because of the large number of animals required for our experimental setup, we started the first replicate of all treatments on the 13th strobilation day and completed the additional replicates during the following days, resulting in a total of ten harvesting days. Each incubation was conducted for 7 days and the last replicate was started on the 22th day.

Experimental design and carbonate chemistry

Since the positive effect of food can cover up potential adverse effects of temperature increases in *A. aurita* ephyrae (Båmstedt et al. 2001), we conducted our study in the absence of food, which is more representative of the natural winter conditions during strobilation (Lucas 2001; Holst 2012). We developed a full-factorial (temperature (two levels), oxygen (three levels), pCO_2 (six levels)) experimental design consisting of 36 treatments, and five replicates each. The acidification and deoxygenation treatments were realised by bubbling the experimental vessels with mixtures of specialty gases (pCO_2 and O_2, basi Schoeberl GmbH & Co. KG, Rastatt, Germany). Ephyrae were exposed to six different pCO_2 levels (van Vuuren et al. 2011; IPCC 2013) : i) 400 ppm as present-day pCO_2; ii) 800 ppm as 2100 RCP6.0 projection; iii) 1000 ppm as 2100 RCP8.5 pCO_2 projection; iv) 1500 ppm as an intermediate value; v) 2000 ppm as 2300 RCP8.5 pCO_2 projection; and vi) 4000 ppm as an extreme value, result of a combination of increased CO_2 in hypoxic/eutrophic systems and future OA conditions (Melzner et al. 2013; Wallace et al. 2014). Oxygen treatments were established at three different levels of oxygen saturation (20 %, 10 % and 5 % DO), 20 % DO representing natural percentage of oxygen in the air (current conditions in the North Sea) and 5% DO approaching coastal hypoxia thresholds (≤ 2 mg O_2 L^{-1} (Rabalais et al. 2010). Two different temperature treatments were used for this experiment based on boreal autumn-winter temperatures, when the strobilation process starts (e. g. Hernroth and Gröndahl 1983):

current winter temperature for Helgoland surrounding waters (5 °C), and simulated autumn temperature considering a projected end-of-century increase of 2 °C (15 °C) (Wiltshire and Manly 2004; IPCC 2013).

t_0	
pH	8.01
Salinity	32.4
Temperature (°C)	10
TA (µmol L^{-1})	2380
Silicate (µmol L^{-1})	8.66
Phosphate (µmol L^{-1})	4.21
Nitrite (µmol L^{-1})	1.05
Nitrate (µmol L^{-1})	22.82
NOx (µmol L^{-1})	23.87
Ammonium (µmol L^{-1})	8.23

Table IV-1: Initial conditions in the filtered (0.2 µm) sea water from the North Sea**.** Water was stored and analysed at 10 °C before being used for gas treatments at 5 and 15 °C. TA = total alkalinity; NOx = nitrogen oxides.

Filtered (0.2 µm) North Sea water (see Table IV-1 for initial conditions) was actively bubbled with the different combinations of CO_2 and O_2 through same-size glass tubes in 100 mL Erlenmeyer flasks (filled up till ~110 mL to reduce air-water gas exchange) covered with parafilm. The experiment was conducted in two temperature-controlled rooms (5 and 15 °C, respectively), therefore temperature remained constant during the experimental time. After 24 h of active bubbling, pH and oxygen had reached the desired values in both temperature treatments, so gas supply was reduced to ~4 bubbles sec^{-1} (~0.2 mL air sec^{-1}). Subsequently, the ephyrae were added to the flasks. This bubbling ensured the maintenance of the desired experimental conditions and kept the ephyrae in the water column while avoiding any damage to the organisms. Oxygen and pH were measured with handheld devices (WTW Oxi 315i and WTW pH 315i; accuracies: ± 0.5 % of the measured volume and ≤ 0.005 pH ± 1 digit, respectively). Samples for total alkalinity (TA) were taken by non-pyrogenic sterile filtration (Sartorius; 0.2 µm) and stored in 100-mL brown glass bottles at 5 °C. Potentiometric titration was conducted at room temperature always in technical duplicate with a titration unit, connected to an automatic sample changer (Titroline alpha plus, SI Analytics, Germany, pH

0.0 to 14.0 ± 0.02) with an average precision of ± 10 µmol kg^{-1} (Kranz et al. 2010). Both titration unit and automatic sampler were operated via titration controller software (TitriSoft 2.72). The carbonate system was calculated from TA, pH, temperature and salinity using CO2Sys (Lewis et al. 1998) through CO2calc (Robbins et al. 2010) for initial conditions (Table IV-1) and for every treatment at the end of the experiment (Table IV-2). Equilibrium constants of Mehrbach (1973) refitted by Dickson & Millero (1987) were used. Throughout the following text and figures, references are made to the target values of pCO_2 (400, 800, 1000, 1500, 2000 and 4000 ppm, respectively) and oxygen (5, 10 and 20% DO) rather than to the values measured, which are compiled in Table IV-2.

Biological measurements

Carbon content. At every initial harvesting day we collected a subsample of healthy and well-shaped ephyrae (5 replicates, 3 individuals each) to establish initial carbon conditions. This was done in order to control for potential differences among the different cohorts. Furthermore, at the end of the experiment, after seven days, four ephyrae were randomly selected after being filmed, and briefly rinsed with milliQ water to prevent any weight bias from attached salt. Ephyrae were then preserved in pre-weighted zinc cups, dried (60 °C) and weighed on a microbalance (Sartorius SC2; readability = 0.1µg). Vario MICRO cube CHNS analyzer (Elementar) was used to measure carbon content of the ephyrae.

Swimming behaviour. We documented the effect of the combined stressors on the swimming activity by filming each individual *A. aurita* ephyra (n = 900) at the end of the seven days exposure to different treatments following the procedure described by Kikkawa and colleagues (2010). The ephyrae were transferred individually from the experimental vessels to a 100 mL crystallization dish and observed under an Olympus SZX16 stereomicroscope. Each ephyra (five per experimental unit) was filmed for one minute at 7 frames per second with an Olympus DP71 camera connected to the stereomicroscope. We determined the pulsation rate of the marginal lappets by counting only movements in which all eight lappets contracted simultaneously, irrespective of the position of the ephyra in the dish (in the water column or on the bottom). Erratic movements, such as contraction of only a few arms or irregular arm movements were rare and not included in the counts.

Table IV-2: Target and measured values of treatment parameters. Initial values (t_1) before ephyrae were added to the experimental units and values at the end of the experiment (t_7). Recalculated pCO_2 was stablished from averaged total alkalinity (TA), pH, temperature and salinity measurements. Values represent averages and standard deviations of three measurements. Absence of standard deviation indicate only one measurement.

pCO_2 (ppm) target	T (°C)	pH t_1	pH t_7	TA (µmol L^{-1})	Recalculated $pCO2$ (ppm)
400	5	8.13 ± 0.01	8.04 ± 0.06	2420 ± 27	427
800	5	7.90 ± 0.01	7.84	2436 ± 14	710
1000	5	7.82 ± 0.00	7.75 ± 0.04	2445 ± 31	882
1500	5	7.66 ± 0.01	7.53 ± 0.02	2427 ± 17	1501
2000	5	7.55 ± 0.01	7.48 ± 0.04	2451 ± 54	1813
4000	5	7.27 ± 0.01	7.18	2444 ± 29	3456
400	15	8.20 ± 0.01	7.97 ± 0.04	2443 ± 34	531
800	15	7.94 ± 0.01	7.85 ± 0.01	2446 ± 38	718
1000	15	7.86 ± 0.00	7.73 ± 0.08	2456 ± 20	1009
1500	15	7.69 ± 0.01	7.59 ± 0.05	2456 ± 28	1571
2000	15	7.55 ± 0.02	7.50 ± 0.04	2462 ± 28	1750
4000	15	7.28 ± 0.01	7.24 ± 0.10	2430 ± 14	3190

O_2 (% DO) target	T (°C)	mg O_2 L^{-1} t_1	mg O_2 L^{-1} t_7
20	5	7.35 ± 0.09	7.32 ± 0.19
10	5	4.24 ± 0.28	4.74
5	5	2.27 ± 0.32	2.94 ± 0.40
20	15	5.22 ± 0.03	5.58 ± 0.28
10	15	2.99 ± 0.11	3.81 ± 0.22
5	15	2.14 ± 0.13	2.09 ± 0.58

Mortality rates. Activity and condition of the ephyrae inside the flasks were checked on a daily basis. Mortality after the seven-day experimental period was quantified per bottle as a ratio of dead versus initial ephyrae. The total length of the experiment (one week) was chosen

to be able to detect differences in mortality between the treatments, but at the same time ascertaining that enough animals survived to also be able to measure weight and swimming behaviour after the experimental period (Fu et al. 2014).

Data analyses

For each of the three response variables measured (i.e. final carbon content, mortality and swimming activity) generalized linear models were used to analyse the experimental variability, using pCO_2, O_2 concentration, and temperature as explanatory variables. The models included the three-way interaction amongst variables and all the two-way interaction combinations. In order to assess which variable influenced the studied parameters a backward stepwise model selection process was used (Zuur et al. 2009). According to this procedure, the higher level interactions are sequentially removed from the complete model in case they are not significant until only significant terms are left in the model. If the three-way interaction was included in the model this automatically included all variables and two-way interactions, independently of their respective significance levels. In the same fashion, if a two-way interaction is included in the model both individual terms contributing to the interaction were included in the model, too.

All linear models were fitted with the Gaussian family and without previous transformation apart from the analysis of mortality. These models were of the form:

$$y = \alpha + \beta x \, \mathrm{pCO}_2,$$

where y is the independent variable and it is modelled as a linear relationship with pCO_2, and α represents the intercept and β the slope. Temperature and O_2 were treated as categorical variables. These categorical variables modify the intercept either independently or through their interaction. Similarly if an interaction is found between categorical variables and pCO_2, the slope of the linear regression is modified (Zuur et al. 2009).

In order to deal with proportional data (mortality), a logistic regression using the binomial distribution was used. This procedure deals with mortality proportions as probabilities of either survival or death for each case. In logistic regression, the logarithmic odds of an event are modelled as a linear function of the explanatory variables (Zuur et al. 2009)

$$\ln(O_i) = \ln\left(\frac{P_i}{1-P_i}\right) = g(x),$$

where O_i are the odds, P_i the probability of success and $g(x)$ a linear combination of the explanatory variables.

All analyses were performed using R version 3.0.2.

3. Results

The models fitted to initial carbon content showed that this parameter was not related to the treatments (no variable showed a significant effect on initial carbon). Therefore the results presented here should represent the effect of the treatment unbiased by initial size of the ephyrae. As a precaution, initial carbon content was included in the best models to check if it increased the goodness of fit, which it never did.

Carbon content after seven days

The values of the ephyrae biomass strongly differed among ambient conditions (5 °C, 20 % DO, 400 ppm pCO_2) and the most extreme treatment (15 °C, 5 % DO, 4000 ppm pCO_2), being 6.55 ± 1.12 and 3.40 ± 1.64 µg C per individual, respectively (Fig IV-1a-c). This variability was captured by a model with two-way interactions between temperature-O_2 and pCO_2-O_2 which was selected as best fit for the final carbon content data (Table IV-3, Fig IV-2a). Temperature showed a negative effect on carbon content (Fig IV-2a), and this was shown by the negative intercept in the model (Table IV-3, $\alpha_{(T = 15\,°C)}$ = -1.73). The detected temperature-O_2 interaction (Table IV-3, $\alpha_{(T * O2)}$), described how the difference in carbon content caused by temperature varied across O_2 treatments; this difference being smaller the higher the O_2 concentration (Fig IV-2a).

Overall there was a negative relationship between carbon content and pCO_2 (Fig IV-2a, β = -1.34 $*10^{-4}$). The pCO_2-O_2 interaction (Table IV-3, $\beta_{(pCO2 * O2)}$) however, turning it slightly positive at higher O_2 concentrations, i. e. with slightly higher carbon contents at higher O_2 concentrations (Fig IV-2a). Overall the model had a 19.2 % explanation power.

Metabolic demands were established as the difference between initial and final body carbon weights, varying among 2.48 % (at 5 °C) to 4.58 % (at 15 °C) per day (averaged from t_7 measurements) for an initial carbon content of 7.65 ± 1.40 µg C ephyra^{-1}. Potential effects of differences in the initial size of ephyrae were taken into account in this model. The outcome indicated that initial carbon content did not explain final carbon content of the animals.

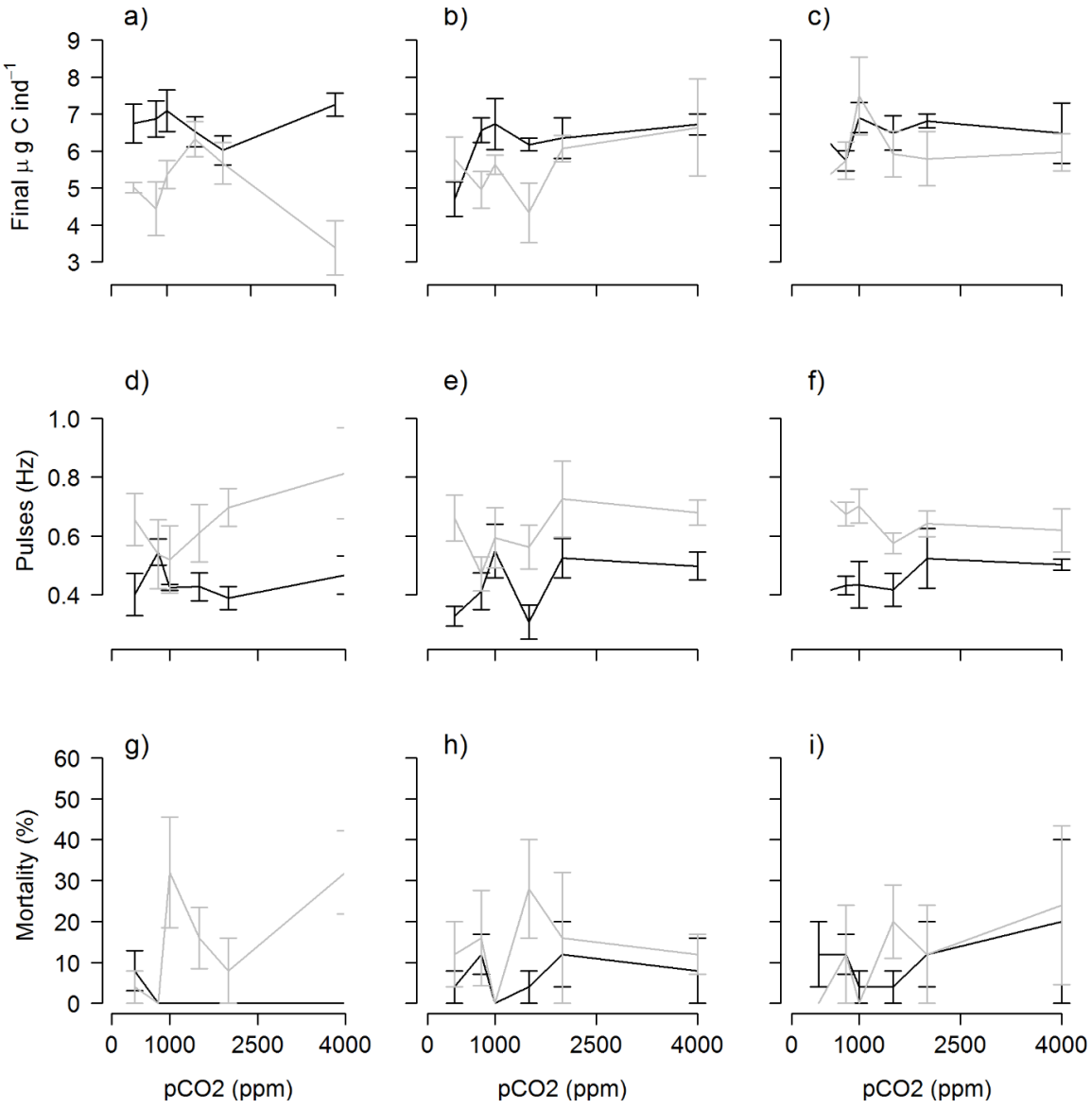

Fig IV-1: Response of *A. aurita* ephyrae under different temperature (5 and 15 °C), pCO_2 (400, 800, 1000, 1500, 2000 and 4000 ppm) and O_2 conditions (20, 10 and 5 % dissolved oxygen (DO)). Biomass (µg C) represented as (a-c) carbon content; (d-f) swimming activity (Hz), and (g-i) mortality (%). Black lines: 5°C; grey lines: 15°C. Error bars indicate standard error of the mean (*n*=5).

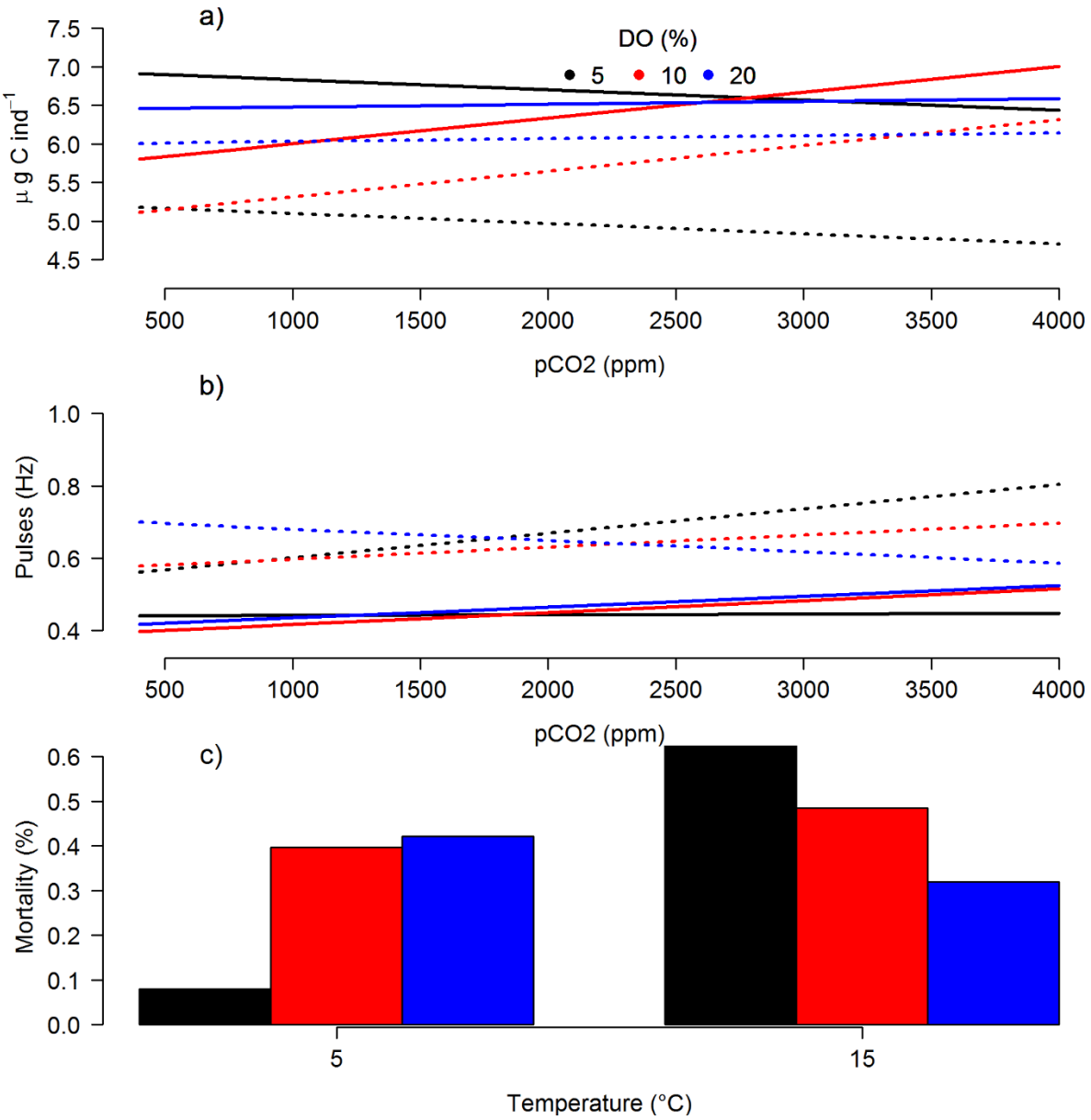

Fig IV-2: Model predictions for *A. aurita* ephyrae (a) carbon content (µg C ind^{-1}), (b) swimming activity (Hz), and (c) mortality (%) under future environmental changes. Statistically significant effects of temperature at 5 ºC (solid lines) and 15 °C (dashed lines), pCO_2 (400, 800, 1000, 1500, 2000 and 4000 ppm) and O_2 conditions (20, 10 and 5 % DO) are represented.

Activity

Ephyrae activity values were lower at ambient conditions (5 °C, 20 % DO, 400 ppm pCO_2; 0.403 ± 0.169 Hz) than at the most extreme treatment (15 °C, 5 % DO, 4000 ppm pCO_2; 0.814 ± 0.347 Hz), as it is shown in Fig IV-1d-f. A three way interaction among pCO_2, O_2 and

temperature was the best fit for the swimming activity data. This indicates that all three stressors exert a combined effect on the activity, but teasing out their individual effects might be complicated. Due to the non-significance of most estimates in this model (Table IV-3) it was difficult to draw clear conclusions.

The combined effect can be visualized on the different slopes of regression lines across temperature and O_2 treatments (Fig IV-2b). At low temperature, the slopes barely differ from 0 (i.e.: slight pCO_2 effect), while at high temperature these slopes clearly differed from 0 (i.e.: strong pCO_2 effect on swimming activity). Furthermore, this pCO_2 effect at high temperature was different across O_2 treatments, ranging from clearly positive at low O_2 concentrations (Table IV-3, $\beta_{(Baseline)}$), to negative at high O_2 concentrations (Table IV-3, $\beta_{(pCO2*O2)}$).

Our model showed how the swimming activity responses to different pCO_2 were very diverse depending on temperature and O_2. The strength of this synergistic effect of the stressors was confirmed by the model, which explained < 30 % of the data variability.

Mortality

Ephyrae mortality was lower at ambient conditions (5 °C, 20 % DO, 400 ppm pCO_2; 12 ± 17.888 %) than at the most extreme treatment (15 °C, 5 % DO, 4000 ppm pCO_2; 32 ± 22.803), as it is shown in Fig IV-1g-i. Mortality was affected by O_2 concentrations, temperature and their interaction, but not by pCO_2 (Table IV-3, Fig IV-2c). The effect of O_2 on mortality was reverse at high and low temperature treatments (Fig IV-2c). Our model showed mortality increased with increasing O_2 concentrations at low temperature, while the opposite happened at high temperature. This model had a low explanatory power ca. 7 %, probably due to the large standard errors of the data (Table IV-3).

Table IV-3: Coefficient estimates of the best fitting models for each variable (β = slope of pCO_2, α = Intercept). The baseline values represent the 5 °C temperature and 5 % O_2 treatment. Both β and α are modified depending on the treatment variables and the interactions among them (e. g. in the case of 5 °C and 20 % O_2, α would be the baseline + α (10% O_2) + any α interactions). Bold faces indicate *p* values < 0.05.

		Estimate	S.E.	*p* value
Carbon Content				
T = 5°C, O2 = 5%	α baseline	6.963	0.349	**<0.001**
T = 5°C, O2 = 5%	β baseline	$-1.34*10^{-4}$	<0.001	0.387
O2 = 10%	α (O2)	-1.292	0.489	**0.009**
O2 = 20%	α (O2)	-0.52	0.495	0.295
T = 15°C	α (T)	-1.728	0.359	**<0.001**
O2 = 10%	β (pCO_2 * O_2)	$4.64*10^{-4}$	<0.001	**0.032**
O2 = 20%	β (pCO_2 * O_2)	$1.68*10^{-4}$	<0.001	0.453
T = 15°C, O2 = 10%	α (T * O_2)	1.042	0.505	**0.041**
T = 15°C, O2 = 20%	α (T * O_2)	1.28	0.505	**0.012**
Swimming activity				
T = 5°C, O2 = 5%	α baseline	0.439	0.052	**<0.001**
T = 5°C, O2 = 5%	β baseline	$2.00*10^{-6}$	<0.001	0.939
O2 = 10%	α (O_2)	-0.056	0.074	0.447
O2 = 20%	α (O_2)	-0.003	0.075	0.646
T = 15°C	α (T)	0.035	0.075	0.21
O2 = 10%	β (pCO_2 * O_2)	$3.11*10^{-5}$	<0.001	0.399
O2 = 20%	β (pCO_2 * O_2)	$-2.09*10^{-6}$	<0.001	0.471
T = 15°C	β (pCO_2 * T)	$7.64*10^{-5}$	<0.001	**0.077**
T = 15°C, O2 = 10%	α (T * O_2)	0.122	0.105	0.409
T = 15°C, O2 = 20%	α (T * O_2)	0.274	0.106	**0.047**
T = 15°C, O2 = 10%	β (pCO_2 * T * O_2)	$-7.48*10^{-5}$	<0.001	0.208
T = 15°C, O2 = 20%	β (pCO_2 * T * O_2)	$-1.41*10^{-4}$	<0.001	**0.02**
Mortality				
T = 5°C, O2 = 5%	α baseline	0.013	0.011	0.212
T = 15°C	α (T)	0.091	0.033	**0.006**
O2 = 10%	α (O_2)	0.053	0.026	**0.04**
O2 = 20%	α (O_2)	0.057	0.027	**0.034**
T = 15°C, O2 = 10%	α (T * O_2)	-0.076	0.048	0.119
T = 15°C, O2 = 20%	α (T * O_2)	-0.108	0.046	**0.021**

In summary, our results showed synergistic effects among pCO_2, temperature and oxygen concentration on the studied ephyrae. Separately, although significant, the effect of our treatments remained small. We observed a negative relationship between pCO_2 and carbon content. At the same time, there was a clear effect of temperature in all studied parameters; warmer treatment (15 ºC) generally led to lower biomass, higher pulsing rates and higher mortality. In turn, mortality was also affected by oxygen depletion alone, reaching higher rates under lower oxygen availabilities, notwithstanding resulting almost zero under the combination of hypoxic and cold treatments.

4. Discussion

This study is pioneering in analyzing metabolic and physiological reactions of a vulnerable life stage of jellyfish species to a wide range of abiotic conditions. We observed a synergistic effect of pCO_2, O_2, and temperature on ephyrae swimming activity and of pCO_2 and O_2, on the final carbon content of the ephyrae. Interestingly, the lowest final biomass was recorded in the most extreme treatment (15 °C, 5 % DO, 4000 ppm pCO_2), but essentially only in this most extreme environment the effect of the stressors was clearly visible. One could argue that this extreme CO_2 treatment is outside any realistic scenarios. However, these values have been reported, especially in conjunction with coastal hypoxia (Wallace et al. 2014). Separately, although significant, the effect of our treatments remained small, especially when end-of-century climate change scenarios are considered (IPCC 2013), which do not include pCO_2 values higher than ca. 1000 ppm. Clearly, even the purportedly most vulnerable stage of *A. aurita*, the ephyra, has a strong resistance against environmental stress when compared to other pelagic taxa (Richardson 2008; Vaquer-Sunyer and Duarte 2008; Purcell 2012; Pitt et al. 2013). One of the explanations to this response could be the great adaptability of both benthic and pelagic forms of *A. aurita* to their environment (Lucas 2001). Another reason for this resistance could be that metabolic rates of ephyrae are relatively low, as Fu and colleagues described in their paper (2014), and we observed in our experimental animals. Thus, low metabolic demands could explain not only the strong resistance to starvation, but also to environmental stressors.

To date, most of our knowledge about ecophysiological reactions of different life stages of scyphomedusae to climate stressors is based on analyses and observations which did not consider starvation as a condition. However, results from experiments conducted under different food supply regimes may differ, and also cover up the effect of the stressors. For instance, scyphozoan polyps from different species have shown a high tolerance to direct effects of low pH (Winans and Purcell 2010), although they may suffer the effect of OA indirectly, through changes in food quality (Lesniowski et al. 2015). Consequently, considering that wild newly released ephyrae have to naturally cope with food scarcity periods, we designed our experiment using starvation as a condition to ascertain that the effects observed were in fact caused by the environmental stressors and not by differences in food uptake.

Although some *in situ* and long-term studies predict a negative influence of warming on *A. aurita* medusae (North Sea, (Lynam et al. 2010)), others indicate no relationship between abundances and climate change (Dutch Wadden Sea, (van Walraven et al. 2015)). We observed that there is a consistent effect of temperature on starved ephyrae, as temperature has interacted in all our models with other factors to explain the physiological and behavioral responses of the animals. Temperature is positively correlated with swimming activity and mortality rates of the ephyrae -characteristics of a higher metabolism- and lower biomass of the young medusa stages. Thus, warmer winter conditions might lead to higher mortality in ephyrae.

Scyphozoans are among the taxa with the highest tolerance to hypoxia, and some life stages even benefit from oxygen depletion; e. g. while fish avoid or die in waters with less than 2-3 mg O_2 L^{-1}, many jellyfish are tolerant to levels lower than 1 mg O_2 L^{-1} (Shoji et al. 2005; Vaquer-Sunyer and Duarte 2008). However, little is known about the effect of hypoxia on physiological reactions of young scyphomedusae. Our results indicate that the biomass of the ephyrae as well as the activity and mortality rates, are influenced by oxygen availability. Further, we found synergistic effects of oxygen depletion and warming on activity and mortality rates. Interestingly, ephyrae reacted to hypoxic-cold treatments with a decrease in activity and mortality rates. A similar behaviour has been previously observed not only in this species (*A. aurita)* but also in *Cyanea capillata* (Kramp 1937; Rasmussen 1973; Hernroth and Gröndahl 1983). Ephyrae are released from the strobilae in autumn and overwinter near the bottom during the cold months before they appear in upper water layers and continue their

development in spring (Kramp 1937; Rasmussen 1973; Hernroth and Gröndahl 1983). This cycle may reflect an energy-saving mechanism to survive low temperatures and oxygen depletion by reducing activity. Thus, the reduced activity we observed in hypoxic-cold treatments might be some sort of a dormancy response of the ephyrae.

Negative effects of massive jellyfish occurrences on economically relevant activities have been reported worldwide during the last decades, such as impacts on fisheries, aquaculture, power plants and tourism. These have moved scyphomedusae into a research field of special interest. Not only deoxygenation (as noted above), but also warming (Richardson 2008 and the references therein) and acidification (Fabry et al. 2008) could benefit jellyfish as they are more detrimental to competitors and predators such as fish than to the gelatinous zooplankton. Species like *A. aurita* which are not only food competitors for resources of zooplanktivorous fish but also predators of early stages of fish larvae and juveniles (Bailey and Batty 1984; Titelman and Hansson 2006; Uye 2011; Acuña et al. 2015) are of particular concern. Consequently, *A. aurita* could take advantage of these human-driven environmental stressors -especially in overexploited ecosystems- and eventually displace fish (Purcell and Arai 2001; Purcell 2005; Purcell et al. 2007). Nevertheless, our knowledge on the competitive interactions from jellyfish and fish reacting to these environmental changes is in fact still too limited to allow robust conclusions, especially on an experimental scale, beyond inferences from field data.

According to our results, environmental changes predicted by the end of the century (ocean acidification, warming and deoxygenation, according to IPCC 2013) should not affect the scyphozoan *A. aurita* in a substantial way. This species may however not be robust to larger changes in these stressors, especially if simultaneous increases in atmospheric pCO_2 levels and seawater temperature occur. However, making general predictions about *A. aurita* blooms is challenging since (i) environmental requirements differ among the benthopelagic metagenetic cycle (planulae-polyps-ephyrae-adults); (ii) ephyrae from different latitudes might have different thermal windows for growth and survival (Gambill and Peck 2014; Pascual et al. 2014); (iii) experimental designs between published studies may differ; and (iv) multiple stressors studies for the different life stages are still lacking. Further studies based on the effect of climatic stressors on early stages (both polyps and ephyrae) of different *Aurelia* spp populations are still needed for a better understanding of these species in a

climate change context. Hence, multiple stressor research is of paramount importance to reach a more complete understanding and to be able to evaluate global change effects, especially for the still unstudied gelatinous zooplankton.

Acknowledgements

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5. SYNOPTIC DISCUSSION

This thesis illustrates that increased CO_2 may cause indirect bottom-up effects on copepods, which show species- and stage-specific responses to OA. This species-specificity was also observed in hydromedusae within a natural plankton community. Moreover, this thesis also illustrates the synergistic effects of pCO_2, when acting in combination with temperature and oxygen concentration. Thus, within the common context of zooplankton responses to future climate change, three main aspects were investigated here:

1. elevated pCO_2 effects on natural plankton communities from boreal and subtropical systems,

2. direct and indirect pCO_2 effects on grazers (*O. marina, A. tonsa*), and

3. effects of multiple climatic stressors (acidification, warming, deoxygenation) acting simultaneously on *A. aurita* ephyrae.

Ocean acidification may affect marine organisms either directly (i.e. by changes in pH) or indirectly (via trophic pathways). The studies described in CHAPTER I and II included both kind of effects combined, while CHAPTER III differentiates between direct and indirect effects and CHAPTER IV focuses on direct pCO_2 effects combined with other climatic stressors.

Throughout the following pages, the results analysed separately in the previous chapters are discussed in a broader context, focusing on the OA effects on plankton communities, copepods and jellyfish, respectively. Moreover, the implications and perspectives for future climate change research on zooplankton are included.

- **OA effects on natural plankton communities**

Nutrient conditions play an important role in the response of plankton communities to OA (Alvarez-Fernandez et al. submitted). Generally, pCO_2 effects seem to be more intense at limiting inorganic nutrient concentrations (Paul et al. 2015; Sala et al. 2015; Bach et al. 2016b). This is because elevated CO_2 levels cause an increase in phytoplankton standing stocks — more pronounced in smaller-sized taxa— and this effect on primary producers may be

transferred differently into heterotroph primary consumers depending on the inorganic nutrient availability (Alvarez-Fernandez et al. submitted). Thus, different responses may be observed in CO_2-enhanced communities depending on the initial nutrient conditions.

The responses of plankton communities to OA were studied in two mesocosms experiments. The first one was a long-term mesocosms experiment in a boreal system (Gullmar Fjord KOSMOS2013), which allowed us to study the influence of high CO_2 on an entire winter-to-summer plankton succession. The second one (Gran Canaria KOSMOS2014) was a mesocosms experiment in an oligotrophic system which allowed us to investigate how OA impacts might differ between oligotrophic conditions and phases of high biological productivity. Before we can compare these results and put them into the context of previous similar experiments, I will briefly recapitulate the main results of both experiments:

During the Gullmar Fjord KOSMOS2013 study (Fig 5.1A), the first phytoplankton bloom was fuelled by inorganic nutrients upwelled during winter and enclosed in the mesocosms at the beginning of the study (Bach et al. 2016b). Nutrient depletion occurred during the first phytoplankton bloom, and a second phytoplankton bloom developed directly after the first one collapsed, most likely fuelled by remineralized nutrients (Bach et al. 2016b). Before the first phytoplankton bloom, potential food items for copepods consisted mainly of phytoplankton between 5 and 40 µm and microzooplankton biomass below 2 µg C L^{-1} (Horn et al. 2016b; Taucher et al. 2017b). During the second bloom, the entire mesocosms system was dominated by *Coscinodiscus concinnus* and the nanophytoplankton fraction (Taucher et al. 2017b), both largely outside the food spectrum of *Pseudocalanus acuspes*, the dominant copepod in the mesocosms. No pCO_2 effect on ciliates abundances or biomass was observed (Horn et al. 2016b), likely responding to a trophic cascade effect caused by the copepodites (Sommer et al. 2004; Calbet and Alcaraz 2007). These may have exerted a top-down control on the microzooplankton population, masking the possible pCO_2 effects on ciliates. However, microzooplankton biomass alone might not have been enough to supply the whole copepod population. The higher copepod abundances under the high-pCO_2 treatment likely responded to a community CO_2-driven bottom-up effect (Rossoll et al. 2012; Schoo et al. 2013; Cripps et al. 2016), depending on higher primary production (Eberlein et al. 2017) and higher chl*a* levels under high-pCO_2 (Bach et al. 2016b). The most plausible explanations for the decay in copepod abundance towards the end of the experiment are that 1) a potential downward

migration towards the sediment traps searching for food sources, 2) the level of top-down control through herring larvae was different, with higher predation pressure in high-pCO_2 mesocosms (Sswat et al. submitted), and 3) it was the end of the season for this species, as evinced by the fact that also in the fjord the densities declined.

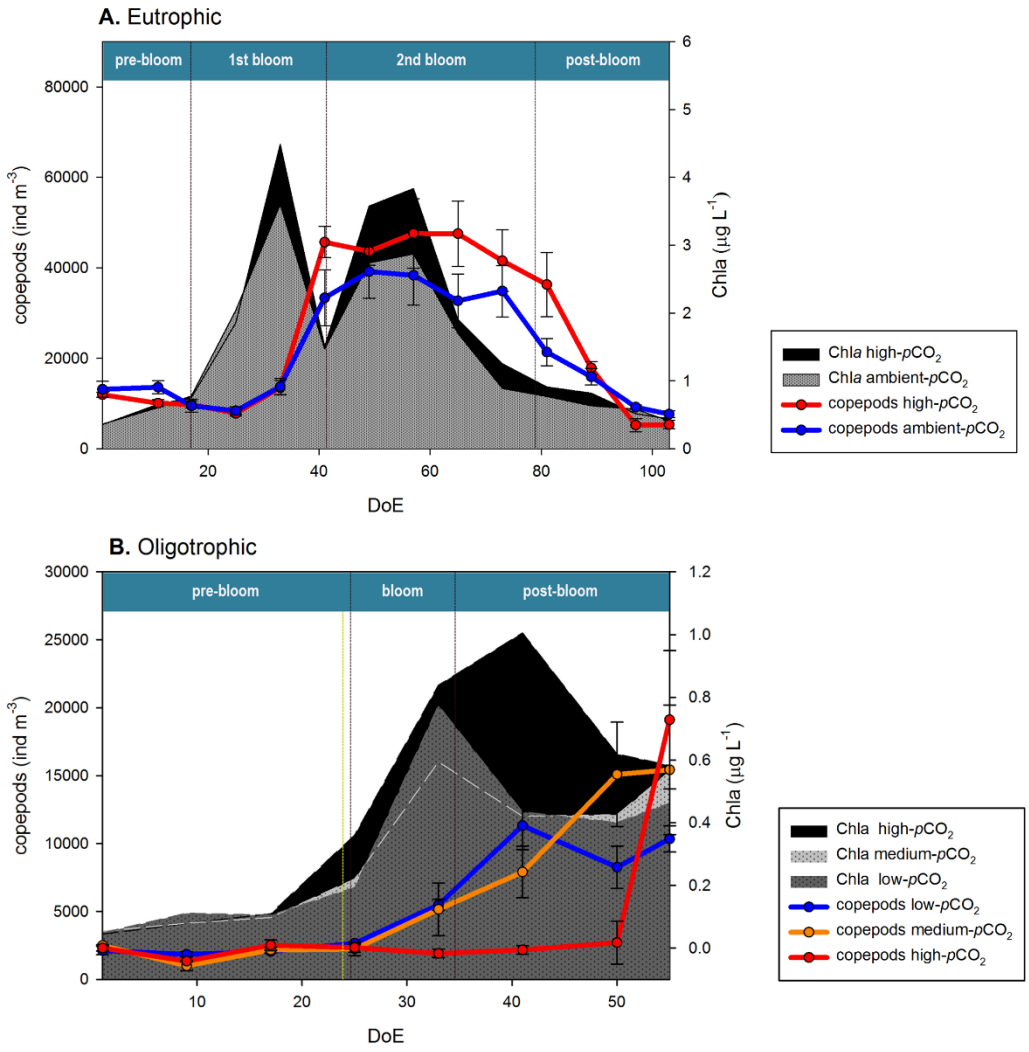

Fig 5.1: Copepod community responses to phytoplankton bloom in A) eutrophic (Gullmar Fjord KOSMOS2013) and B) oligotrophic systems (Gran Canaria KOSMOS2014). Copepod abundance (ind m^{-3}) in the low- (blue), medium- (orange) and high-pCO_2 (red) treatments. Grey fields show Chlorophyll *a* concentrations from HPLC analysis in µg L^{-1} at the different pCO_2 treatments. Error bars represent the standard error and vertical dotted lines the experimental phases of both experiments. Four phases characterised Gullmar Fjord experiment (A): a pre-bloom (until day 16), 1st phytoplankton bloom (day 17-40), 2nd phytoplankton bloom (day 41-79) and a post-bloom phase (from day 80 until the end of the experiment). During Gran Canaria experiment (B) the addition of deep water on day 24 (yellow line) simulated a bloom, which lasted until day 35 in low- and medium- , and until day 47 in high-pCO_2 treatment, respectively. DoE = day of experiment.

In conclusion, CO_2 had an effect on the plankton succession within the studied eutrophic system. Copepod built-up occurred after the first phytoplankton bloom —when inorganic nutrients in the water were depleted— and, during the second bloom and the beginning of the post-bloom phase, copepod abundances were higher under the high-pCO_2 conditions (Fig 5.1A). Thus the CO_2-enhanced increase in autotrophs standing stocks (Chl*a*) travelled up the food web, benefiting heterotrophic consumers such as copepods (CHAPTER I) as well as higher trophic levels (Sswat et al. submitted).

So, the hypothesis that nutrients play a fundamental role in the reaction of systems to CO_2 was tested in the Gran Canaria KOSMOS2014 study (Fig 5.1B), where a bloom was simulated by the addition of deep water. This allowed us to compare the plankton community responses to OA in the nutrient-deplete and nutrient-replete phases within the oligotrophic system. During the first few weeks of the experiment, we observed typical oligotrophic conditions in the mesocosms. Concentrations of all inorganic nutrients were very low and relatively constant (Taucher et al. 2017a). The autotrophic community was expected to experience an increase in biomass (Gismervik et al., 2002) responding to the nutrient input created by the deep water addition. However, under the same nutrient enrichment conditions, a significant effect of CO_2 on plankton succession was observed during this experiment, suggesting that phytoplankton boost was likely faster under high-pCO_2 (Taucher et al. 2017a). These different phytoplankton situations depending on the pCO_2 treatment were in turn reflected by changes in zooplankton community development during the second half of the experiment. Thus, the simulated upwelling caused a phytoplankton bloom and subsequent pronounced differences in succession patterns and food-web structure under high CO_2 conditions. The bloom was dominated by large, chain-forming diatoms (Taucher et al. 2017a). There was a second and smaller phytoplankton bloom in the high-pCO_2 mesocosms dominated by *Vicicitus globosus* (Dictyochophyceae), identified by Riebesell et al. (Riebesell et al., in prep). Harmful or non-edible for zooplankton, it seems likely that the abundance of *V. globosus* caused adverse effects on the plankton community (Chang, 2015) thus preventing the phytoplankton standing stock to reach consumers in the high-pCO_2 mesocosms until the bloom of this alga decayed (~t48).

We could not detect major differences between treatments on copepod abundance during the pre-bloom phase (Fig 5.1B). However, after the simulated upwelling, the plankton

community under high-pCO_2 conditions evolved differently from the low- and medium-pCO_2 mesocosms. Thus, in bloom and post-bloom conditions, elevated pCO_2 might promote higher zooplankton abundances by bottom-up effects of CO_2-enhanced primary production. These pCO_2-fuelling effects would reach grazers and travel up throughout the food web, increasing the transfer of energy to copepods and higher trophic levels (CHAPTER II).

Overall, our results from both eutrophic and oligotrophic studies showed that pCO_2 levels predicted by the end of the century may cause an (indirect) positive effect on copepods in natural plankton communities when primary production is enhanced by elevated pCO_2 levels (CHAPTERS I and II). These results differ from previous plankton community studies on natural coastal communities from the Arctic (Suffrian et al. 2008; Aberle et al. 2013; Niehoff et al. 2013; Hildebrandt et al. 2016) and the Baltic Seas (Horn et al. 2016a; Lischka et al. 2017) which mostly reported on the tolerance of zooplankton to elevated CO_2 concentrations. Most plausible reason to explain the discrepancies in the zooplankton responses could be that mesocosms experiments mentioned above might have been too short to detect changes in life cycles of dominant mesozooplankton species from such cold areas, as noticed by Niehoff et al. (2013). As a comparison focused on copepods, the life cycle of the Arctic *Calanus hyperboreus* is two to four years (Hirche 1997), while *P. acuspes* from the Baltic would produce one generation per year (Renz and Hirche 2006). Thor and Dupont (2015) needed 137 days to ensure the maturity of a second generation of *P. acuspes* females in their experiment in the Gullmar Fjord, and our mesocosms (KOSMOS2013) conducted in the same site lasted for 103 days, ensuring at least a generation. Even though it was shorter (55 days), a response to OA was also detected on copepods during the Gran Canaria KOSMOS2014, since tropical and subtropical copepods have been characterized by having several generations a year (Kimmerer 1983; Hidalgo et al. 2005).

The increase in copepod recruitment observed under elevated pCO_2 conditions during both mesocosms experiments points at pCO_2-induced effects on primary producers under nutrient-replete conditions, which could travel up the food web reaching secondary consumers in both eutrophic and oligotrophic systems (CHAPTERS I and II). Hence, increasing copepod abundances were detected in the experiments when inorganic nutrient levels (NOx) in the water decreased after fuelling the phytoplankton bloom. Copepod might have thus benefitted of OA within CO_2-fueled communities, responding to the CO_2-driven increases in

phytoplankton and microzooplankton standing stocks. These indirect impacts through trophic interactions were expected, since OA may change the biochemical composition of primary producers that affects nutritional food quality for consumers (Rossoll et al. 2012). During both Gullmar Fjord and Gran Canaria studies (Bach et al. 2016b; Taucher et al. 2017a) a significant effect of CO_2 on plankton succession was observed, thus suggesting that phytoplankton boost was likely faster under high-pCO_2. This situation could in turn cause a CO_2-dependant reduction in trophic efficiency during bloom phases, due to the limited capacity of micro- and mesozooplankton grazers to use the boosted phytoplankton production (Calbet et al., 2014). The result would be a more-autotrophic and less-efficient food web under high pCO_2 conditions when the consumers mismatch the phytoplankton bloom (Calbet et al., 2014;Cripps et al., 2016), as observed in Gran Canaria KOSMOS2014 study during the second bloom in high-pCO_2 mesocosms (CHAPTER II).

- **OA effects on copepods**

Despite the fact that copepods have been traditionally considered as tolerant to end-of-century pCO_2 scenarios, responses to OA observed in this group seem to be species- and stage-specific, and depend on the community trophic interactions. However, some general patterns can be established for a better understanding of OA effects on copepods.

The slowed-down development observed in *A. tonsa* nauplii and copepodites (CHAPTER III) agree with previous studies where early life stages were described as the most sensitive, pointing to a potential negative effect on survival and/or development (e.g. Mayor et al. 2007; Cripps et al. 2014a). However, a positive CO_2 effect was observed in *P. acuspes* copepodites during the Gullmar Fjord KOSMSO2013 mesocosms experiment (CHAPTER I). These contrasting responses likely mirror the differences in food source between laboratory experiments and natural plankton communities, since direct pH effects on consumers seem to be of lesser importance that the associated decrease in food quality (CHAPTER III). Hence, copepods might benefit of realistic end-of-century pCO_2 levels, where CO_2-driven increases in phytoplankton and microzooplankton standing stocks after bloom events may cause an increment in copepod abundances (CHAPTER I and II). However, pCO_2 effects could also be detrimental when copepod feeding is limited to a single food source whose quality is

diminished by a decrease in C:nutrients in the algae caused by the excess of CO_2 in the water (Schoo et al. 2013). This negative effect of pCO_2 was observed in egg production and females' metabolism (Thor and Dupont 2015; Thor and Oliva 2015) as well as in the slowed-down developmental rates from calanoid nauplii and copepodites (CHAPTER III). These results thus suggest that the potential decrease in copepod food quality under elevated pCO_2 might cause indirect effects via trophic pathways on marine food webs, unless copepods could compensate the deficiencies in the food quality by selecting foods which most closely match their metabolic needs. Similar responses were detected in *Daphnia* fed with high CO_2 cultured algae (Urabe et al. 2003; Urabe and Waki 2009): while a decrease in growth rates was observed when feeding on a monospecific algae, this effect was dampen when feeding on a mixed algae, despite lowered C:nutrients in the algal diets. This imply that algal diets composed of multiple species can mitigate the adverse effects of elevated CO_2 on herbivore performance (Urabe and Waki 2009).

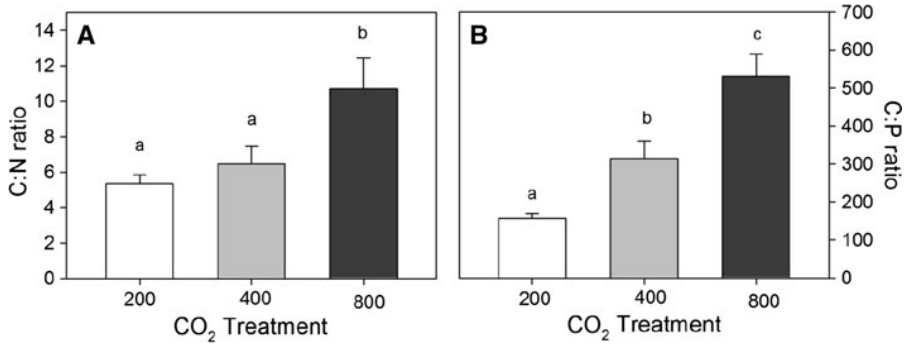

Fig 5.2: Stoichiometric measures of *R. salina* under three different pCO_2 treatments (200, 400 and 800 ppm). A) molar C:N, B) molar C:P. Both C:nutrients ratios increase with pCO_2. Statistical differences ($p < 0.05$ Tukey's honest significant difference (HSD) test) are indicated by letters. Error bars indicate standard deviation. *N*=13 per treatment. (Schoo et al. 2013).

Previous laboratory studies suggest that calanoid copepods have a high buffering capacity against projected OA for the year 2100 and beyond (Kurihara and Ishimatsu 2008; Weydmann et al. 2012; McConville et al. 2013). The results presented here, however, show a positive response of two natural populations of calanoids to OA effects. Calanoid copepods were the most abundant during both plankton community studies presented here. During the study

conducted in eutrophic waters (CHAPTER I), *P. acuspes* copepodites were significantly more abundant in the high-pCO_2 treatment (~760 µatm). Secondary production in *P. acuspes*, however, did not respond to high-pCO_2 but followed a temporal trend, with higher clutch sizes and nauplii abundances responding to higher phytoplankton concentration (Chl*a*) and microzooplankton biomass. As in *P. acuspes* copepodites, a positive response to high-pCO_2 treatment was observed in calanoid copepods (adults and copepodites) from the studied oligotrophic system towards the end of the experiment (CHAPTER II). These copepods resulted to be positively affected by medium- and high-pCO_2 levels (~566 and 837 µatm, respectively) responding to phytoplankton and microzooplankton boost. The most plausible explanation for the higher calanoid abundances under the elevated pCO_2 treatments in both systems is a community CO_2-driven bottom-up effect since, in both cases, copepods reacted positively to the CO_2-enhanced plankton succession. Thus, community interactions would have amplified the pCO_2 effects, what could not be observed in the laboratory experiments mentioned above.

Different sensitivities to OA might also be related to copepod habitats. Hence, copepod species which are more exposed to natural pH fluctuations —as vertical migrators or coastal species— would be more tolerant (Lewis et al. 2013; Almén et al. 2014). During this thesis work, responses to OA on females' physiological and reproductive condition were studied in a coastal (*P. acuspes*, Calanoida) and an oceanic system (*Oncaea* sp., Poecilostomatoida). *P. acuspes* (copepodites) and *Oncaea* sp. were both more abundant under high-pCO_2 conditions, however females responded differently to CO_2-driven succession. *P. acuspes* females showed no pCO_2 effect on any of the physiological and reproductive parameters investigated (respiration, carbon content, prosome length, clutch size, hatching success) (CHAPTER I). Nevertheless, high-pCO_2 caused smaller *Oncaea* sp. females, as well as a higher number of immature females and a lower number of egg-carrying mature females, resulting in a clear negative effect at high-pCO_2 on *Oncaea* potential offspring (CHAPTER II). Thus, despite the higher abundances of both species observed under high-pCO_2, OA seem to have a negative effect on *Oncaea* sp. future generations, while *P. acuspes* offspring might be tolerant to pCO_2 increases. The differences in the habitats of both copepods might explain these different responses to OA, considering the natural fluctuations that a copepod would experience in its life time in a fjord versus the environmental stability in an oceanic system. These results

however call for multigenerational studies on copepods, with prolonged pCO_2 exposure times to take adaptive responses into account and discern how the responses to end-of-century pCO_2 levels described here could affect future generations in both copepod species.

- **OA effects on jellyfish**

The connection between jellyfish blooms (scyphomedusae, hydromedusae, siphonophores and ctenophores) and anthropogenic climate change still remains unclear (e.g. Condon et al. 2012; Purcell 2012) although most of the studies suggest that there is a clear anthropogenic effect on coastal environments that may support jellyfish proliferations in the future (reviewed in Purcell et al. 2007). The effects of changing seawater carbonate chemistry on planktonic gelatinous species have been rarely tested, but all results on different gelatinous zooplankton groups —schyphomedusa ephyrae (Kikkawa et al. 2010; Winans and Purcell 2010), coelenterate records (Richardson and Gibbons 2008)— have traditionally pointed to the tolerance of jellyfish to future changes in pCO_2. However, differences within gelatinous groups must be considered in order to understand global change effects on jellyfish. During this thesis, I have focused on studying indirect effects of OA on hydromedusae (CHAPTER I), as well as direct effects of multiple climatic stressors on scyphomedusae (CHAPTER IV).

Results showed that tolerance to OA cannot be generalized since it seems to be rather species-specific, as observed in scyphozoan polyps (Lesniowski et al. 2015) and hydromedusae (CHAPTER I). Thus, during the KOSMOS2013 mesocosms experiment, hydromedusae responses to pCO_2 were different for the two studied species, and while *Hybocodon prolifer* abundance decreased, *Aglantha digitale* was positively affected (CHAPTER I). Given the fact that *A. digitale* —as all hydromedusae but Anthomedusae and most scyphomedusae — has calcium-based structures (statoliths) implied on equilibrium reception, this species is of special interest in order to understand potential OA effects on gelatinous zooplankton. To the best of this author's knowledge, results presented in CHAPTER I of this thesis represent the first study about the effects of OA on hydromedusae to date. Our results suggest that hydromedusa statoliths might not be a pCO_2-target, at least in terms of

hydromedusae abundance. Further ecophysiological analyses, however, are still required for these and other hydromedusae species to confirm this hypothesis.

Sensitivity to OA on gelatinous zooplankton will depend on the interactions with other environmental stressors such as warming and deoxygenation, since these factors may occur together in coastal regions globally. Thus, despite of the tolerance of *A. aurita* ephyrae to end-of-century pCO_2 scenarios (IPCC 2013), this species may not be robust to larger changes in OA, warming and deoxygenation, especially if increases in atmospheric pCO_2 and sea water temperature occur simultaneously (CHAPTER IV). Thus e.g. we observed that ephyrae biomass strongly differed among ambient conditions (5 °C, 20 % DO, 400 ppm pCO_2) and the most extreme treatment (15 °C, 5 % DO, 4000 ppm pCO_2). The synergistic effects observed among pCO_2, temperature and oxygen concentration on the *A. aurita* ephyrae condition highlight the importance of multiple stressors studies in order to make a robust evaluation of future climate change effects.

The tolerance or resilience of jellyfish to climate change is especially important in an ecosystem context when it is compared to the tolerance of other taxa in their same trophic level, such as fish. In fact, jellyfish abundance have been often positively correlated with warm temperatures and low forage fish populations (Purcell 2012, and the references therein). Jellyfish in general may be more tolerant to OA (Fabry et al. 2008) and low DO than fish (Vaquer-Sunyer and Duarte 2008), what may give jellyfish an adaptive advantage over fish in eutrophic environments (Vaquer-Sunyer and Duarte 2008; Purcell et al. 2013). For example, low DO concentrations have been shown to reduce the escape ability of fish larvae, thereby increasing their vulnerability to predation (Purcell et al. 2013). Decreased light penetration may also alter the trophic interactions to benefit non-visual gelatinous predators over visually feeding fish in scenarios such as fjords, where visibility may be reduced due to darkening and eutrophication (Eiane et al. 1999; Purcell 2012), especially where DO concentrations are diminished (Aksnes et al. 2009).

- **Implications for higher trophic levels**

Small planktonic copepods link phytoplankton and protozooplankton with higher trophic levels such as fish and jellyfish (Suchman and Sullivan 2000; Moyano et al. 2009), hence a

positive pCO_2 effect on this major zooplankton components could have a crucial impact on the transfer of energy within the system. This potential pCO_2-effect on tertiary consumers may be conditioned not only by copepod abundance (i.e. food quantity), but also by food quality, since indirect OA effects can be expected to reach copepods by changing the nutritional quality of their prey (Rossoll et al. 2012; Schoo et al. 2013). Some studies have shown the dampening of pCO_2 effects on single species in coastal communities that normally experience high natural fluctuations in pCO_2 (Rossoll et al. 2013; Bermúdez et al. 2016). Our results however showed that trophic interactions within complex coastal plankton communities might also lead to the amplification of pCO_2 effects, resulting in higher abundances of copepods as a response to CO_2-enhanced phytoplankton and microzooplankton standing stocks. This increase in copepod abundance might ultimately benefit fisheries (CHAPTER I, (Sswat et al. submitted)) although further multigenerational and nutritional analyses are still required to discern which will be the quality of copepods as prey in the future when phyto- and microzooplankton biomass are CO_2-enhanced.

Jellysfish are infamous because they can occur in large numbers, which may in turn cause detrimental effects on human activities such as tourism —by stinging swimmers—, fishing —by clogging nets—, aquaculture —by killing fish in net-pens— and power plants —by clogging cooling-water intake screens— (Purcell et al. 2007; Purcell 2012). They also cause negative indirect effects on fisheries by feeding on zooplankton and ichthyoplankton, thereby acting both as predators and competitors of fish (Purcell et al. 2007). Ironically, not only anthropogenic climate change but also many human activities such as overfishing and habitat disruptions may contribute to increases in jellyfish populations in coastal waters (Purcell et al. 2007; Purcell 2012). Several correlations show inverse biomasses of jellyfish and forage fish, probably because of reduced competition for zooplankton when forage fish are depleted (Purcell 2012). Thus in overexploited areas jellyfish have been reported to exceed the biomass of fish, causing a profound ecosystem change that might have possible consequences from carbon cycling to fish stock recovery (Lynam et al. 2006). Moreover, in addition to competitors of jellyfish, many of their predators are being removed either intentionally (as for Scombridae and other fish commercial species) or accidentally (as for sea turtles that are caught in nets or longlines (Arai 2005; Purcell 2012). Jellyfish proliferations may also be enhanced by constructions in coastal waters such as aquaculture farms, docks, marinas, breakwaters, wind

farms, etc. which provide hard surfaces that strengthen polyps settlement (Holst and Jarms 2007; Purcell 2012). Thus, considering the resilience observed in hydro- and scyphomedusae to climate change (CHAPTER I and IV), jellyfish blooms might burst into future ocean more frequently than nowadays, unless the trend in global climate change and human activities as the mentioned above turn into a more sustainable pace.

- **Future research**

It has been lately claimed from different authors the necessity of scaling up from individuals or species to ecosystems (e.g. Queirós et al. 2015; Riebesell and Gattuso 2015), as well as the combination of manipulative experiments, field observations and modelling to understand climate change (Guinotte and Fabry 2008). However, the variety of biological responses— both competitive and synergistic— at the organism and population level might prevent extrapolation to the community and ecosystem level (Rossoll et al. 2013). Therefore, the reader might find that the results presented in this thesis show disparities between the outcome from individuals and community studies: if individuals (e.g. calanoid copepods) show negative responses to OA when studied in the laboratory (CHAPTER III) but a positive response when studied in communities (CHAPTER I and II), which is the valid conclusion? On the one hand, laboratory experiments are not representative of real situations in the ocean since they do not reflect the complexity of the interactions within the community. On the other hand, natural communities studies do not allow to separate out direct and indirect pCO_2 effects to understand the physiological mechanisms behind the zooplankton responses to OA, and laboratory experiments would permit to do that. It seems thus necessary to combine both OA community studies with laboratory experiments to get more solid conclusions about climate change effects on zooplankton. Hence, going back to the example of the OA effects on calanoid copepods, the study presented in CHAPTER III: (1) shows that copepods did not suffer from pH changes but from indirect pCO_2 effects and (2) illustrates the physiological and metabolic responses of copepods to CO_2 when there is only a food source available. The close-to-natural condition would be represented by a natural plankton community, where copepods could generally chose the most convenient food (as those from CHAPTERS I and II), but we could not know if OA effects could be direct or indirect. Cheaper and less challenging to develop than community studies —especially when it comes to multiple stressors studies—

laboratory experiments have traditionally formed the broad base of the OA research. However, future research should consider to rather focus on the effects of climate change on communities to make predictions, since the outcome based on single species experiments does not reflect the manifold and complicated interactions within communities.

To this end, mesocosms studies are convenient for outdoor close-to-natural conditions experiments in complex ecosystems, allowing the consideration of pCO_2 perturbations all-over entire communities (Riebesell et al. 2008). Moreover, the multidisciplinary approach in mesocosms experiments allow a broader view of OA effects in plankton communities. This allows us to analyse OA effects on mesozooplankton combining quantitative and taxonomical analyses (CHAPTERS I and II) with other methodologies such as imaging —e.g. ZooScan (Taucher et al. 2017b) and KielVision (Taucher et al. in prep.)— or particle flux analyses (Stange et al. submitted).

Notwithstanding the suitability of mesocosms experiments, replicability is complicated due to the patchiness of the plankton communities and differences in the initial conditions, hence initial effects of unresolved ecophysiological variables can propagate (Riebesell et al. 2008). Thus, variability within the planktonic communities existing when mesocosms are closed (e.g. abundances from the different groups, differences in the physiological conditions of the patched communities), may perpetuate and increase due to biological interactions all along the experiments. When such kind of uncertainties amplify, high standard deviations can be generated -even between replicates within the same treatment-, masking potential pCO_2 effects. Although recently uncertainty quantification model-based studies have been conducted for primary producers (Moreno de Castro et al. 2017), there are yet no models for zooplankton that allow us to understand the effect of initial variability in consumers when studying OA effects in a mesocosms approach. These tools would be extremely useful for future mesocosms studies in order to solve replicability problems associated to zooplankton distribution in natural communities.

The combination of laboratory and mesocosms studies in plankton communities during BIOACID I and II (including those presented here) has provided the basis for extensive modelling approaches and meta-analyses during the final phase of the BIOACID project (BIOACID III). The objective will be to synthetize data and make useful conclusions that allow

ecosystem managers, policy makers and general public to understand the consequences of ocean acidification effects on global ocean under end-of-century IPCC scenarios, and take appropriate steps to minimize CO_2 emissions in the near future.

6. CONCLUSIONS & OUTLOOK

The focus of this thesis was to investigate whether there is a direct link between responses to OA in communities and single organisms, focusing on copepods and jellyfish. We investigated CO_2-driven changes on zooplankton communities from different marine ecosystems in two large scale mesocosm studies. One study was performed in a Swedish fjord (Gullmar Fjord), and the other in the oligotrophic subtropical Northeast Atlantic off Gran Canaria Island. Additional laboratory experiments on copepods and jellyfish were conducted for a better understanding of the tolerance of these two taxa to future climatic scenarios.

The main conclusions of this thesis work can be summarized as follows:

1. pCO_2 levels predicted by the end of the century may cause an (indirect) positive effect on copepods in natural plankton communities when primary production is enhanced by elevated pCO_2.

During Gullmar Fjord KOSMOS2013 Expedition we observed that plankton succession responded to high pCO_2 by an increase in Chl*a* (Bach et al. 2016b) and primary production (Eberlein et al. 2017), ultimately benefiting copepod abundances under high-pCO_2 conditions. This was especially noticeable in the copepodite stage of the calanoid *P. acuspes*, which was the most abundant species in the copepod-dominated mesozooplankton community. The higher copepod abundance under high-pCO_2 conditions finally resulted in higher herring survival of herring larvae (Sswat et al. submitted).

A similar pattern in zooplankton was observed during Gran Canaria KOSMOS2014 Expedition after a simulated bloom event. Based on this study, elevated pCO_2 levels are not expected to cause major effects on zooplankton communities under oligotrophic conditions in pre-bloom phases. However, during bloom phases, end-of-century pCO_2 levels may promote higher zooplankton abundances by bottom-up effects of CO_2-driven increases in phyto- and microzooplankton standing stocks. Hence, pCO_2-fuelling effects may reach grazers and travel up throughout the food web, increasing the transfer of energy to copepods and higher trophic levels.

This positive OA effect on secondary production was by somehow unexpected, based on previous mesocosms studies on natural coastal plankton communities in the Arctic (Suffrian et al. 2008; Aberle et al. 2013; Niehoff et al. 2013) and the Baltic (Lischka et al. 2015; Horn et al. 2016a), which mostly reported on a tolerance of zooplankton towards high CO_2 concentrations, or only subtle changes in the community. However, a positive effect of pCO_2 was detected on copepod-dominated communities, which might have benefitted of pCO_2-induced effects on primary producers under nutrient-replete conditions. Our findings suggest that the increase in copepod abundances in such CO_2-driven trophic cascade may have important implications for future fisheries and ecosystem services.

2. OA may cause indirect negative pCO_2 effects on consumers through a decrease in food quality when having only a food source, while direct pH effects seem to be of lesser importance.

Although it has previously been suggested that smaller organisms should be more affected by ocean acidification (Flynn et al. 2012) no direct effect of seawater pCO_2 were observed on dinoflagellates and copepods. Due to environmental variability (e.g. upwelling), diapause at depth, and ontogenetic development during ascent from great depths, many zooplankton (including larval stages) already experience pH levels well below what is predicted for surface waters in year 2100 (Olson and Kawaguchi 2011). Thus, predicted changes in surface seawater pH may be small relative to the range of pH zooplankton experience during their lifespan. These organisms could already be well adapted to seawater pH variations and potential effects of hypercapnia.

When grazers cannot compensate the deficiencies in the food quality by selecting foods which most closely match their metabolic needs, the CO_2-driven decrease of primary producer's quality may negatively affect zooplankton growth (*O. marina*) and development (*A. tonsa* nauplii and copepodites). On the contrary, when dinoflagellates and copepods can feed on natural plankton communities enhanced by CO_2, we observed positive pCO_2 effects on dinoflagellates growth rates (Horn et al. 2016b) as well as zooplankton abundance (CHAPTERS I and II, Taucher et al. 2017b). As in community experiments it is not possible to separate out

the pCO_2 direct and indirect effects, it seems necessary to combine both community studies and laboratory experiments in order to gain a deeper understanding of consumers' sensitivities to OA and the consequent effects on future zooplankton populations and trophodynamics.

3. Responses to OA are species-specific both in copepods as well as in hydromedusae

Different copepod sensitivities as well as the amplification of the pCO_2 effects after the phytoplankton bloom were detected in both mesocosms experiments. For example, in oligotrophic conditions, the trend in temporal responses to OA in Calanoida and Poecilostomatoida was different, despite that both orders responded positively to increased pCO_2 (CHAPTER II). Hence, Poecilostomatoida abundances were higher in high-pCO_2 conditions before nutrient-enrichment, while Calanoida only reacted after CO_2-enhanced phyto- and microzooplankton standing stocks increased. Responses to elevated pCO_2 depended also on the life-stage of the individuals, copepodites generally being the most sensitive stage (CHAPTER I). In order to implement these results, further long-term community studies on CO_2-enhanced copepod populations will be important to discern whether some copepod species may benefit from OA in the future.

Species-specific sensitivity of hydromedusae to OA was shown for the first time in this study (CHAPTER I). *H. prolifer* (Anthomedusa) reacted negatively to high pCO_2 by lower abundances, while *A. digitale* (Trachymedusa) was more abundant in the high-pCO_2 treatment. This result was by somehow unexpected, given the fact that *A. digitale* have statoliths, i.e. calcium-based structures that could be a target for lower pH (as Richardson and Gibbons (2008) also noted), therefore affecting equilibrium. Our findings suggest that hydromedusae with statoliths are not necessarily more sensitive than those without these calcium-based structures, and consequently hydromedusa statoliths might not be sensitive to OA, at least in realistic end-of-century scenarios. Further ecophysiological analyses yet are still required for these and other hydromedusae species to confirm this hypothesis.

4. The scyphomedusa *A. aurita* is not likely to be affected by end-of-century pCO_2 levels in a substantial way.

This species, however, may not be robust to larger changes in OA, warming and deoxygenation, especially if simultaneous increases in atmospheric CO_2 and seawater temperature occur. Thus, further studies based on the effect of climatic stressors on early stages of different *Aurelia* spp populations are still needed in order to implement our understanding of *A. aurita* sensitivity to global change. Since zygotes and early embryonic stages -which lack specialized ion-regulatory epithelia- may be especially sensitive (Melzner et al. 2009), multiple stressors experiments on *A. aurita* benthic-pelagic coupling from planulae to ephyrae will be determining to disentangle the role of jellyfish in the future ocean.

General outlook

Major components of mesozooplankton communities might be resilient, or even benefit from elevated pCO_2 levels when grazers can do compensatory feeding. Accordingly, in natural communities, copepods abundance under OA scenarios might increase as a response to pCO_2-induced effects under nutrient-replete conditions, as observed in both eutrophic and oligotrophic systems. Thus, since copepods serve as major food source for fish as well as jellyfish, CO_2-driven trophic cascades as the ones described here might have important implications for future fisheries and ecosystem services.

As in community experiments it is not possible to separate out the pCO_2 direct and indirect effects, it seems necessary to combine both community studies and laboratory experiments to gain a deeper understanding of consumers' sensitivities to OA and the consequent effects on future zooplankton populations. Thus, the simulation of future conditions in natural plankton communities becomes of striking importance to make solid predictions about zooplankton responses to global change. Accordingly, it seems meaningless to investigate responses of single organisms to single stressors given that this does not simulate real situations in the future ocean. Thus, future research should consider to focus on the

conjunction of community and multiple environmental stressors approaches. This way we could better understand the consequences of ocean acidification on plankton communities within a more realistic global change context.

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1. **Algueró-Muñiz M**, Alvarez-Fernandez S, Thor P, Bach LT, Esposito M, Horn HG, et al. Ocean acidification effects on mesozooplankton community development: results from a long-term mesocosm experiment. PLoS One. 2017;12(5):e0175851. doi: 10.1371/journal.pone.0175851.

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4. Taucher J, Haunost M, Boxhammer T, Bach LT, **Algueró-Muñiz M**, Riebesell U. Influence of ocean acidification on plankton community structure during a winter-to-summer succession: An imaging approach indicates that copepods can benefit from elevated CO_2 via indirect food web effects. PLoS One. 2017;12(2):e0169737. doi: 10.1371/journal.pone.0169737.

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IN REVIEW OR IN PREPARATION

1. Sswat M, Stiasny M, Taucher J, **Algueró-Muñiz M**, Jutfelt F, Clemmesen C, et al. Indirect effects of ocean acidification on growth and survival of herring larvae. *In prep*.

2. Stange P, Bach LT, Taucher J, Boxhammer T, Krebs L, **Algueró-Muñiz M**, et al. Ocean acidification induced food web changes slow down degradation of sinking particles in an upwelling-stimulated oligotrophic plankton community. Front Mar Sci. *Submitted*.

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4. **Algueró-Muñiz M**, Horn HG, Alvarez-Fernandez S, Spisla C, Aberle-Malzahn N, Bach LT, Guan W, Achterberg E, Riebesell U, Boersma M. Impacts of ocean acidification on the development of a subtropical zooplankton community during oligotrophic and simulated bloom conditions. *In prep.*

5. Taucher J, Stange P, **Algueró-Muñiz M**, Bach LT, Nauendorf A, Kolzenburg R, et al. In situ camera observations of particle size spectra during an upwelling-induced plankton bloom reveal influence of zooplankton on marine snow formation. *In prep.*

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~ ~ ~

ERKLÄRUNG

Erklärung gemaß § 6 (5) der Promotionsordnung der Universität Bremen für die mathematischen, natur- und ingenieurwissenschaftlichen Fachbereiche vom 14. März 2007

Hiermit erkläre ich, María Algueró-Muñiz, dass ich die Arbeit mit dem Titel:

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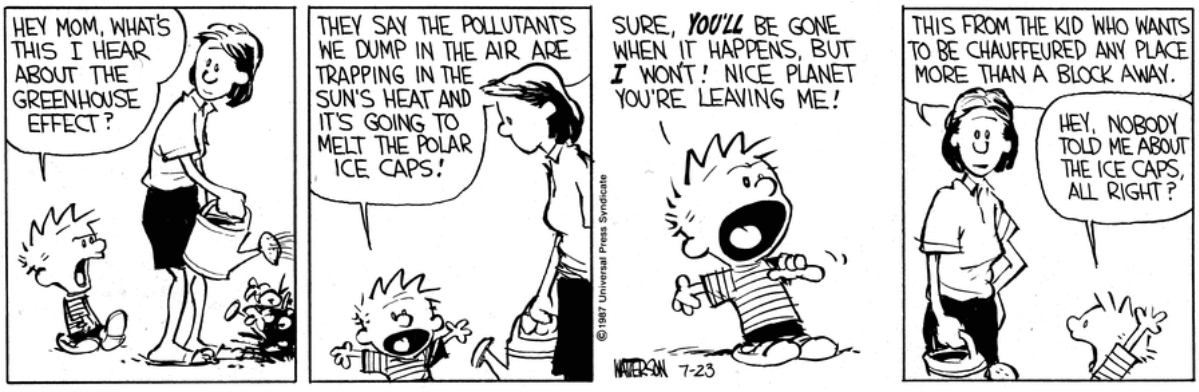
María Algueró-Muñiz


HEY MOM, WHAT'S THIS I HEAR ABOUT THE GREENHOUSE EFFECT?
THEY SAY THE POLLUTANTS WE DUMP IN THE AIR ARE TRAPPING IN THE SUN'S HEAT AND IT'S GOING TO MELT THE POLAR ICE CAPS!
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SURE, YOU'LL BE GONE WHEN IT HAPPENS, BUT I WON'T! NICE PLANET YOU'RE LEAVING ME!
WATTERSON 7-23
THIS FROM THE KID WHO WANTS TO BE CHAUFFEURED ANY PLACE MORE THAN A BLOCK AWAY.
HEY, NOBODY TOLD ME ABOUT THE ICE CAPS, ALL RIGHT?