Physical and Physiological Growth Constraints of Key, North Sea Gelatinous Zooplankton

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Dissertation

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Contents

Summary

The motivation of this thesis was the present scarcity of knowledge regarding juvenile stages of gelatinous zooplankton and the controversial discussion around whether global occurrence of gelatinous zooplankton is rising or not. To understand the development of jellyfish blooms it is necessary to investigate all stages of their complex life cycles, especially the metagenic life cycles. The aim of this thesis was to investigate the physical and physiological growth constraints of gelatinous zooplankton, focusing on under-studied juvenile stages of key North Sea gelatinous zooplankton: the scyphozoan benthic polyp; and the planktonic ephyra stage. The thesis focused on climate-driven factors that are predicted to change due to global warming. We conducted tri-trophic food chain experiments to research the direct and indirect effects of OA and changes of nutrient availability. Polyp growth and carbon content was not affected by CO_2, but was significantly negatively affected by P-limitation of the food. Therefore, the conclusion is that phosphorus can be a limiting factor influencing the fitness of scyphozoan polyps and that phosphorus limited food is of poor nutritional quality. The next step was to investigate the effect of temperature and different levels of CO_2 on *C. capillata* polyps and *Ch. hysoscella* ephyrae. The aim was to determine how rising temperature and CO_2 influence the survival and development of juvenile scyphozoan stages. The growth and carbon content of polyps and ephyrae were significantly affected by temperature, but once again there was no effect either by CO_2 or by the interaction between temperature and CO_2. The results confirm that increasing temperatures may have a positive effect on polyps of *Ch. hysoscella* but a negative effect on *C. capillata* ephyrae and that increasing CO_2 concentration up to 1000 ppm will not affect the development of the investigated juvenile stages. Data sets gained in laboratory experiments provide closed system results, and it would be necessary to conduct field experiments for a complete picture. Studies by e.g. Holst and Jarms (2007) state that scyphozoan polyps prefer artificial hard-substrate for settlement and polyp development but it is difficult to find colonies in the field. To investigate the effect of current, polyps on plates were tested in an annular flow channel under different current velocities. Additionally, two field experiments were conducted to verify the laboratory results and survey the influence of water current and shelter on the survival of scyphozoan polyps. The laboratory and field results demonstrate that current velocity has a negative effect and protection a positive effect on the survival of scyphozoan polyps. Increasing anthropogenic influences around our coast provide new hard-substrate and protected sites for polyps, supporting the distribution of gelatinous

zooplankton. It is important to mention that a generalized conclusion for gelatinous zooplankton would only be possible once all species at all developmental stages have been investigated.

The conclusion of this thesis is that the juvenile stages of the species used for this work have a wide range of tolerance for the investigated abiotic factors. This makes them more adaptable to the predicted shift of climate. The survival and reproduction of the juvenile stages controls the formation of blooms, especially for the benthic polyp stage of the scyphozoan metagenetic life cycle.

Zusammenfassung

Die Motivation dieser Arbeit war die aktuelle spärliche Wissenslage in Bezug auf die Jugendstadien von gelatinösem Zooplankton und die kontroverse Diskussion, ob das globale Vorkommen von diesem ansteigt oder nicht. Um die Entwicklung von Quallenblüten zu verstehen, ist es notwendig, jedes Stadium eines komplexen Lebenszyklus zu untersuchen, besonders bei den metagenetischen Lebenszyklen. Das Ziel dieser Arbeit war es, die physikalischen und physiologischen Wachstumsfaktoren der Schlüsselvertreter des gelatinösen Zooplanktons in der Nordsee zu untersuchen mit einem Fokus auf die wenig untersuchten Jugendstadien von Scyphozoen, das benthische Polypen- und das pelagische Ephyra-Stadium. Die Arbeit befasst sich mit klimaabhängigen Faktoren, die sich den Vorhersagen nach aufgrund von globaler Erwärmung ändern werden. Wir haben Nahrungskette-Experimente über drei trophische Ebenen durchgeführt, um die direkten und indirekten Effekte der Ozeanversauerung und Veränderungen in der Nährstoffverfügbarkeit zu untersuchen. Das Wachstum und der Kohlenstoffgehalt von Polypen wurden durch CO_2 nicht beeinflusst, jedoch wurde Wachstum und Kohlenstoffgehalt signifikant negativ von Phosphorlimitation in der Nahrung beeinflusst. Deshalb ist die Schlussfolgerung, dass Phosphor ein limitierender Faktor sein kann, der die Fitness von Scypho-Polypen beeinflusst und dass phosphorlimitierte Nahrung eine geringere Nahrungsqualität in Bezug auf Nährstoffe hat. Im nächsten Schritt wurde der Effekt von Veränderungen der Temperatur und verschiedenen Konzentrationen von CO_2 auf Polypen von *Ch. hysoscella* und Ephyren von *C. capillata* untersucht. Das Ziel war es, herauszufinden, wie steigende Temperatur und CO_2 das Überleben und die Entwicklung von Jugendstadien von Scyphozoen beeinflussen. Wachstum und Kohlenstoffgehalt von Polypen und Ephyren wurden durch Temperatur signifikant beeinflusst, jedoch gab es zum wiederholten Male keinen Effekt, weder bei CO_2 noch bei der Interaktion von Temperatur und CO_2. Die Ergebnisse bestätigen, dass steigende Temperaturen einen positiven Einfluss auf Polypen von *Ch. hysoscella* haben können, jedoch einen negativen Effekt auf Ephyren von *C. capillata* und dass steigende CO_2-Konzentrationen bis zu 1000 ppm keinen Einfluss auf die Entwicklung der untersuchten Jugendstadien haben. Datensets, die in Laborexperimenten gewonnen werden, liefern Ergebnisse für geschlossene Systeme, aber es ist wichtig, Feldexperimente durchzuführen, um ein komplettes Bild zu bekommen. Untersuchungen von z.B. Holst und Jarms (2007) sagen aus, dass Scypho-Polypen künstliche Hartsubstrate zur Besiedelung und Entwicklung bevorzugen, aber es ist schwierig, Kolonien im Freiland zu finden. Um den Effekt von Strömung zu untersuchen, wurden Polypen auf Platten in einem ringförmigen Strömungskanal

verschiedenen Strömungsgeschwindigkeiten ausgesetzt. Zusätzlich wurden zwei Freilandexperimente durchgeführt, um die Laborergebnisse zu bestätigen und den Einfluss von Wasserströmung und Schutz auf das Überleben von Scypho-Polypen zu untersuchen. Die Labor- und Freilandergebnisse beweisen, dass Strömungsgeschwindigkeit einen negativen Effekt und Schutz einen positiven Effekt auf das Überleben von Scypho-Polypen hat. Die zunehmenden anthropologischen Einflüsse auf unsere Küsten bieten neues Hartsubstrat und Schutz für Polypen, was die Ausbreitung von gelatinösem Zooplankton fördert. Es ist wichtig zu erwähnen, dass eine generelle Aussage zum gelatinösen Zooplankton nur dann möglich ist, wenn alle Arten und alle Stadien untersucht werden.

Das Ergebnis dieser Dissertation ist, dass Jugendstadien der Arten, die hier untersucht wurden, eine breite Toleranz gegenüber den untersuchten abiotischen Faktoren haben. Das macht sie anpassungsfähiger für die vorhergesagten klimatischen Veränderungen. Der Erfolg von Überleben und Reproduktion der Jugendstadien kontrolliert die Blütenbildung der gelatinösen Zooplankter (Scyphozoa), besonders für das benthische Polypenstadium des metagenetischen Lebenszyklus.

General introduction

Gelatinous zooplankton, including jellyfish (cnidarians), ctenophores (comb jellies), and pelagic tunicates (the invertebrate chordates that include salps, doliolids and pyrosomes) (Condon et al. 2012), is a group of organisms with a body plan characterized by a high water and low carbon content. This group evolved 500 to 540 million years ago (Richardson et al. 2009) and there is no historical/prehistoric data presenting their distribution and frequency of occurrence over this period. There is currently a debate as to whether populations of gelatinous zooplankton are increasing globally (Brotz et al. 2012; Condon et al. 2012; Condon et al. 2013). Members of this group, especially the scyphozoa, are identified as key members of oceanic ecosystems (Hamner et al. 1975; Mills 1995; Purcell 2007) but very little is known about their behaviour, physiology and the environmental factors influencing their reproduction and survival.

In particular, the role of scyphozoa in food webs is unknown, and whether or not jellyfish are a trophic end of nutrient transport is a subject of debate. The reason is that, especially during blooms, large quantities of carbon (C), nitrogen (N) and phosphorous (P) are fixed by primary producers and consumed by secondary producers, which are then consumed by jellyfish. At this point the carbon is not available for higher trophic levels, because jellyfish are not readily consumed by other predators (Condon et al. 2011). Additionally, jellyfish produce huge amounts of carbon-rich dissolved organic matter. This carbon is used by bacteria, inhibiting their growth and changing the composition of the bacterial community (Condon et al. 2011). Additionally, within blooms, jellyfish have a significant, direct impact on marine food webs. It is documented that gelatinous predators exert top-down control of zooplankton, withdrawing resources for other consumers and therefore limiting secondary production. Hanson et al. (2005) recorded that life expectancy for several species of zooplankton in the Danish Limfjord was halved due to predation by the moon jellyfish, *Aurelia aurita*. One third of the zooplankton standing stock was consumed by jellyfish in the eastern shelf of the Bering Sea (Brodeur et al. 2008). Aside from the fact that jellyfish are competitors with planktivorous fish for food (Lynam et al. 2005a), they prey on marine fish in their early life stages (Purcell et al. 1994). Predation by gelatinous zooplankton can reduce cohorts of newly-hatched larval herring (*Clupea harengus*) (Moller 1984; Purcell and Grover 1990). The importance of jellyfish for food webs is indisputable, but further investigation is necessary to understand factors influencing their appearance, distribution and bloom occurrence.

Below, this introduction will continue with a closer look at a controversial point in the basic discussion: whether gelatinous zooplankton is currently increasing or whether it is a regular increase consistent with a recurring pattern on a larger timescale. The presentation will include some examples of studies supporting individual opinions. This is followed by a description of the impact of a jellyfish bloom for different economic sectors and the introduction of the main factors that regulate jellyfish bloom formation. This leads to a closer characterisation of the factors and the organisms investigated in this thesis. At the end of this introduction, the structure of this thesis is outlined. The goal of this thesis is to investigate the following questions:

- How does pCO_2 / pH affect the growth dynamic of scyphozoan polyps?
- How does nutrient limitation in prey items, especially phosphorus limitation, and food quantity affect the growth of polyps?
- How does pCO2 / pH in combination with temperature affect the growth of the scyphozoan polyp and ephyra stage?
- How do currents affect the performance of scyphozoan polyps in laboratory and field experiments?

Discussions about gelatinous zooplankton, especially jellyfish, are always accompanied by the question as to whether jellyfish populations are increasing globally or not. Brotz et al. (2012) complement the low number of quantitative time series of jellyfish abundance, usually carried out as part of scientific surveys, with non-conventional information to evaluate trends of jellyfish abundance after 1950. Bortz et al. used the Large Marine Ecosystem (LME) framework, which defines regions/areas based on ecological factors instead of economic or political criteria (Sherman and Alexander 1986; Sherman and Hempel 2008), to present a positive trend in jellyfish abundance. 28 (62%) of 45 analysed LMEs show an increasing trend, while only 3 LMEs show a decreasing trend (Brotz et al. 2012) (Fig 1).

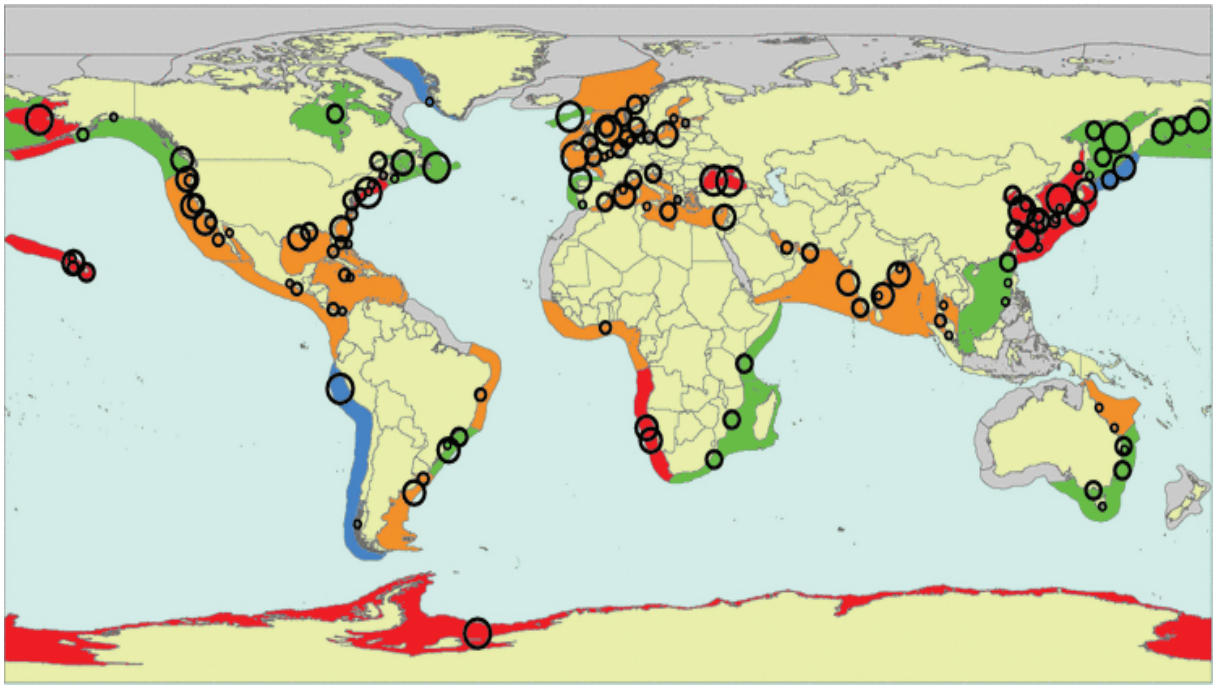

Figure 1: Map regions where jellyfish are increasing. The colors indicate different degrees of development: red (high increase), orange (low certainty), green stable/variable, blue decrease, grey no data available. (Brotz et al. 2012)

The abundance of jellyfish has also increased in the North Sea (Lynam et al. 2005a), and this trend is predicted to continue (Attrill et al. 2007). The assumption of a global increase in jellyfish in the ocean is supported by data analysis, where data from an upper-layer plankton-monitoring program (CPR) from 1958 until 2000 were analyzed. Modelling of jellyfish frequency for seven different predicted global warming scenarios shows a mean increasing trend of jellyfish abundance until end of the 21st century in the North Sea (Fig 2).

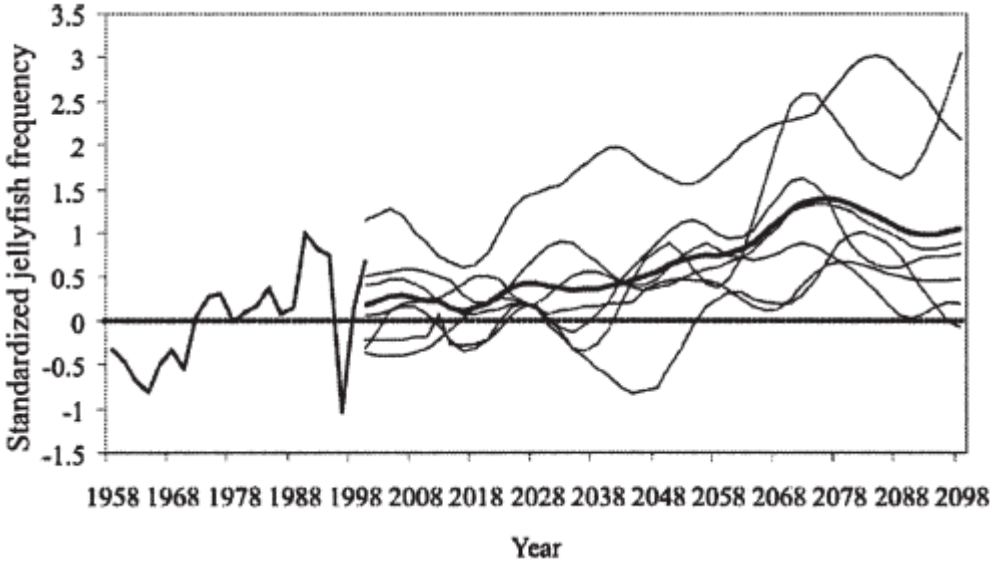

Figure 2: Predicted trends in jellyfish frequency in west-central North Sea until 2100. Thick black line represents mean for the seven global warming scenarios. (Attrill et al. 2007)

Jellyfish blooms are defined as an increase of gelatinous biomass (Lucas and Dawson 2014). Jellyfish blooms can have a huge impact on many economic sectors, e.g. tourism, power supply industry, aquaculture, and fisheries (Graham 2001; Ramsak and Stopar 2007; Lo et al. 2008). Due to media presentations of some jellyfish – e.g. the Australian stinger (*Chironex fleckeri*, Cubozoa), the Portuguese Man -of-War (*Physalia physalis*), or the Lion's Mane (*Cyanea capillata*) – as "the terror of the sea" (El Mundo/El Dia de Baleares, Sunday June 16, 2013), people are horrified when they see a jellyfish in the water. Due to lack of knowledge, tourists are deterred when a mass occurrence of jellyfish dominates swimming beaches, even when the

species present is not hazardous. The resulting turnover losses due to decreasing tourism has a negative economic effect on the touristic sector in coastal regions (Lucas et al. 2014). The increase in biomass and aggregation of large numbers of jellyfish during a bloom also affects the power supply industry localised along coasts by clogging cooling system pipes (Lucas et al. 2014). To avoid overheating, whole plants must be shut down, leading to costs for the operating company. Events like this have been recorded in the USA, Japan, England, Sweden and Israel. Jellyfish blooms also have an effect on the aquaculture sector (Lucas et al. 2014). Stinging jellyfish in high abundance around aquaculture farms can injure the skin and gills of fish reared in cages when the jellyfish pass through the mesh in whole or in part (Båmstedt et al. 1998). Indirect effects are caused by de-oxygenation of the surrounding water (Baxter et al. 2011a; Baxter et al. 2011b). Mass occurrence of the mauve stinger, *Pelagia noctiluca*, in the North Channel of the Irish Sea in 2007 led to the death of over 200,000 salmon with a value of 1 million Euro (Doyle et al. 2008).

Organisms

Adult medusae of *Cyanea lamarckii*, *Cyanea capillata* and *Ch. hysoscella* occur in high abundance during summer months in the German Bight, North Sea, with a highest mean abundance of 1.8 ± 2.7 ind. 100 m^{-3} (Russel 1970; Hay et al. 1990; Barz and Hirche 2007). The fourth investigated species, *A. aurita,* and a fifth species, *Rhizostma octopus* (Linneaus, 1788), do not occur every year. The annual cycle of seasonal appearance starts with the appearance of *C. lamarckii* from February until August, followed by *A. aurita* (if appearing) and *C. capillata* from April to August, and *Ch. hysoscella* from end of July to September (Barz and Hirche 2007). Systematically all four species belong to the phylum Cnidaria and the class Scyphozoa. Specific to the phylum Cnidaria is the complex life-cycle of many species. The metagenetic life cycle alters between the sexually reproductive planktonic medusa and the asexually reproductive benthic polyp. Fertilized eggs develop in the brood pouches of the mature medusae into free swimming planula larvae. Planktonic planula larvae settle preferably downwards on selected appropriate substratum and metamorphose to a polyp. In response to environmental triggers, the polyp produces the juvenile medusae stage (ephyrae) by transverse fission, a process called strobilation. Planktonic ephyrae develop into adult sexually reproductive medusae, closing the scyphozoan metagenetic life cycle. Data concerning the ecology and

survival of juvenile medusa stages are rather scarce. Considering the high number of planula released by a single female medusa, and the number of ephyra produced per polyp, it is likely that juvenile benthic stages have a considerable influence on jellyfish success.

The main factors, as described in the literature, that regulate jellyfish bloom formation are global warming, eutrophication, and overfishing. Global warming is changing environmental factors like salinity, temperature, solar irradiance, and pH with significant effects on marine organisms. The significant negative effects of rising temperatures on jellyfish populations have already been investigated by Purcell and Decker (2005) and Brodeur et al. (2008). Eutrophication based on upwelling processes or river inflows is a cyclical natural process in the marine ecosystem, but the human induced impact on that process has increased dramatically since the beginning of the industrial revolution in the second half of the 18th century. Additional nutrient flow into the nutrient cycle – caused by sewage, deforestation, the usage of fertilizers on the adjoining land, and the displacement of reactive nitrogen emitted to the atmosphere during fossil fuel combustion (Purcell and Arai 2001) – is changing the nutrient composition. The effects of eutrophication have been associated with a biomass increase of jellyfish (Arai 2001). Gelatinous zooplankton, especially jellyfish, benefit from this human induced eutrophication due to increasing phytoplankton production and the consequential increase of large and small zooplankton, the main food source for jellyfish. Simultaneously there is balanced competition for food resources, such as zooplankton, between jellyfish and many fish species. Overfishing of predatory fish stocks has affected marine ecosystems across the world (Scheffer et al. 2005; Baum and Worm 2009). Collapse of low-trophic-level planktivorous fish stocks competing with jellyfish for resources has led to drastic changes in the entire food web and is characterized by a high biomass of jellyfish (Mills 2001; Roux et al. 2013).

Nutrients

Eutrophication leads to a change of nutrient concentration in marine habitats that can influence the capacity of productivity and the composition of primary producer communities (Elser et al. 2007). Changes in primary producer communities are influencing predators, as some of their phytoplankton prey is higher quality food than others (Irigoien et al. 2000; Malzahn and Boersma 2009). Furthermore, changes in nutrient concentration also affect the nutrient composition of phytoplankton. These changes in phytoplankton nutrient composition alter the quality of the phytoplankton as food for consumers (Sterner et al. 1998; Schoo et al. 2010). These changes in the body nutrient composition of organisms at the lowest trophic level of the food web can be transported to higher levels where they influence consumer fitness (Malzahn

et al. 2007; Boersma et al. 2008; Malzahn et al. 2010; Schoo et al. 2010), but it is not clear if this is also applicable for jellyfish. Nutrient stoichiometry of algae, i.e. the ratio between nutrients (N and P) and carbon, can obviously be affected both by inputs of the macronutrients as well as by the availability of carbon.

Temperature

Currently global average temperatures are increasing, which is probably the biggest threat to marine life at present (IPCC 2014). Every organism has a specific temperature range that allows survival, growth and reproduction. The temperature window for survival is relatively large, but the range is getting narrower for growth, and is smallest for reproduction (Poertner and Peck 2010; Bijma et al. 2013). While it is irrelevant whether the thermal window is between 0 °C and 10 °C or between 25 °C and 45 °C, temperatures beyond the thermal window are lethal. Temperature has an effect on physiological processes for all organisms. Rising temperature leads to faster movement of molecules leading to an acceleration of chemical processes. Following the Van't Hoffsch Regulation, the speed of reaction of physiological processes is doubled when the temperature rises by 10 °C. Where physiological processes are inactivated when temperatures are too low followed by death when frozen, extreme high temperatures also destroy enzymes and proteins leading to the death of the organism. The temperature of the sea surface is predicted to increase between 1.5 to 8 °C above preindustrial values by the year 2300 (Meinshausen et al. 2011). Increasing temperatures, combined with other global warming effects, are already challenging stenothermic species. Some investigations into the effects of temperature on planktonic gelatinous zooplankton exist. Bamstedt (1999) found that temperature has a significant effect on total ingestion, growth rate, growth efficiency, and final body mass of individual ephyrae of *A. aurita.* Fuchs et al. (2014) discovered that on the molecular level the scyphozoan metamorphose process is temperature-regulated. The movement of the cilia of the ctenophore *Pleurobrachia pileus* used for swimming is influenced by temperature (Esser et al. 2004). The newest study published by Algueró-Muñiz et al. (2016) states the robustness of *A. aurita* ephyrae, assuming no detriment to this species' juvenile stage as a result of the predicted changes due to global warming. Only a few studies are available for benthic stages (Brewer and Feingold 1991; Fitt and Costley 1998; Purcell et al. 2012).

Ocean acidification

Ocean acidification (OA) influences critical physiological processes such as respiration and nutrient dynamics, which are important drivers of calcification, ecosystem structure, biodiversity, and ultimately ecosystem health (Guinotte and Fabry 2008). It is another effect of human induced global warming, mainly caused by the increase of the greenhouse gas CO_2, which is vital for life on earth. Beginning with the industrial revolution, anthropogenic emission of CO_2, released by the burning of fossil fuels, deforestation, agriculture, and other human activities, changed the atmospheric CO_2 concentration from a pre-industrial value of 280 ppm to a current value of 400 ppm (Le Quere et al. 2009). OA is expected to continue in the coming decades, even if we reach the targets set by various governments in the COP21 agreement. Predictions in the last IPCC report for atmospheric CO_2 concentrations range from 700-1000 ppm by the end of the twenty-first century (IPCC 2014). Approximately a third of excess CO_2 in the atmosphere will be dissolved in ocean waters, leading to an estimated drop in pH of 0.4 units ($pCO_2 \sim 1{,}000$ ppm) globally by the year 2100 and up to 0.8 units ($pCO_2 \sim 2{,}000$ ppm) by the year 2300 (Caldeira and Wickett 2003; Caldeira and Wickett 2005; IPCC 2014). The increase in oceanic temperature and the acidification of marine environments are occurring simultaneously. This development has significant effects on sensitive marine organisms, especially on organisms dependent on calcification (Melzner et al. 2009; Jansson et al. 2015; Queiros et al. 2015; Steckbauer et al. 2015). Not only are calcifying organisms affected by temperature and acidification but also gelatinous zooplankton (Attrill et al. 2007; Attrill and Edwards 2008; Haddock 2008). It is important to investigate the separate factors individually as well as in combination with each other. It is possible that the significant effects of either temperature or acidification disappear when combined in multifactorial experiments. Or on the other hand, the effect could be intensified when both factors are influencing at the same time.

Water currents

Artificial structures change coastal areas and are constantly proliferating. Protection structures on the coast or offshore wind farms provide new hard substrates for benthic organisms like ascidians and mollusks but also for the benthic stage of the scyphozoan life cycle, the polyps. Besides the creation of new recruitment substrate, the water movement is altered, affecting the organisms. Potential mechanical stress such as water current affects the distribution, growth and reproduction of different algae (Zwerschke et al. 2013; Molis et al. 2015), and could also affect the recruitment, polyp distribution and polyp growth. Very little is known about the effect

of hydrodynamic currents on the survival and/or dispersal of jellyfish, and currents are often underestimated in the context of jellyfish populations (Stenseth et al. 2002; Lynam et al. 2004). For adult planktonic medusae some monitoring data exist from the semi-enclosed Limfjorden (Denmark), where it could be observed that, due to hydrographical incidents, adult medusae of *A. aurita* were washed out of the fjords to open coastal regions (Riisgard et al. 2012a; Riisgard et al. 2012b). This shift from semi-enclosed areas to open coastal waters provided new niches for other species and therefore changed the local community composition. This thesis is a first attempt to investigate the effect of water currents on the survival and performance of the benthic polyp stage in laboratory and field experiments.

For a better understanding as to how the aforementioned abiotic factors influence the distribution of gelatinous zooplankton and bloom formation, it is necessary to investigate the factors individually and in combination for every stage of the metagenetic life cycle. The few available studies investigating the effects of nutrient depletion, temperature, currents, and ocean acidification on jellyfish mainly focus on the adult medusa stage. Therefore, this thesis focused primarily on the juvenile stags of the scyphozoan species.

Outline of the thesis

The main goal of this thesis was to contribute new scientific results on the effects of pCO_2/pH, nutrient limitation, temperature and currents on the growth dynamics of scyphozoan juvenile stages. The results of this thesis should help to understand the physical and physiological growth constraints of gelatinous zooplankton, concentrating on the juvenile benthic stage of Scyphozoa, the polyp stage. The approach of this study was to focus on factors that are predicted to shift due to a changing world climate. Little is known about the effects of abiotic factors on scyphozoan polyps, and even less about the effects of multifactorial stress.

Tri-trophic-experiments were intended to investigate the direct and indirect effects on scyphozoan polyps of OA and changes of nutrient availability due to continuing global warming. The first manuscript is based on two experiments examining the effect of different levels of CO_2 and food quality (experiment 1) and the effect of food quality and quantity (experiment 2) on growth and respiration of the scyphozoan polyps of *C. capillata* and *Ch. hysoscella*.

As a natural consequence of global warming, an increase in water temperature will affect marine organisms directly and indirectly. For the second manuscript, temperature and different levels of CO_2 were tested on *C. capillata* ephyrae and *Ch. hysoscella* polyps. We investigated how increasing temperature and CO_2 concentrations influences the survival and development of juvenile scyphozoan stages.

Laboratory experiment data sets provide information about physiological or physical effects in controlled closed systems, but survival, growth and reproduction in nature is controlled/influenced by a mixture of biotic and abiotic factors. In manuscript three, the effects of water currents on polyps of the scyphozoans *Aurelia aurita*, *Chrysaora hysoscella*, *Cyanea capillata,* and *Cyanea lamarckii* are investigated, both in the laboratory (experiment 1) and in open water (experiment 2 & 3).

The general discussion is a summary of the results of this thesis against a background of a predicted changing ocean due to global warming caused by anthropogenic impact and the non-distinctive effect of this development on juvenile stages of scyphozoa.

Contribution of multiple authors

Chapter I - Effects of food and CO_2 on growth dynamics of polyps of two scyphozoan species (*Cyanea capillata* and *Chrysaora hysoscella*)

Lesniowski, T.J., Gambill, M., Holst, S., Peck, M.A., Algueró-Muñiz, M., Haunost, M., Malzahn, A.M., Boersma, M. (2015)

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Experiments conducted by: Lesniowski, T.J., Gambill, M., Algueró-Muñiz, M., Haunost, M.
Data analyzed by: Lesniowski, T.J.
Manuscript written by: Lesniowski, T.J., Gambill, M., Holst, S., Peck, M.A., Algueró-Muñiz, M., Malzahn, A.M., Boersma, M.

Chapter II - Ocean acidification and temperature affect polyps and ephyrae of the Scyphozoans *Chrysaora hysoscella* and *Cyanea capillata*

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Manuscript written by: Lesniowski, T.J., Malzahn, A.M., Boersma, M., Holst, S.

Chapter III - Effects of water current on the growth and survival of polyps of the scyphozoans *Aurelia aurita, Chrysaora hysoscella, Cyanea capillata* and *Cyanea lamarckii*

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Data analyzed by: Lesniowski, T.J., Goldstein, J., Lüskow, F.
Manuscript written by: Lesniowski, T.J., Holst, S., Goldstein, J., Boersma, M.

Table: Contribution of the author ,Thomas J. Lesniowski, to this thesis in percent [%]

Chapter	Experimental design	Experimental execution	Data analysis	Writing of ms	Preparation of graphs
I	80	90	90	85	100
II	90	80	100	90	100
III	70	60	80	90	100

Chapter I

Effects of food and CO_2 on growth dynamics of polyps of two scyphozoan species (*Cyanea capillata* and *Chrysaora hysoscella*)

Abstract

Increasing anthropogenic CO_2 concentration in the atmosphere is altering seawater carbonate chemistry with unknown biological and ecological consequences. Whereas some reports are beginning to emerge on the effects of ocean acidification (OA) on fish, very little is known about the impact of OA on jellyfish. Especially the benthic stages of metagenetic species are virtually unstudied in this context despite their obvious importance for bloom dynamics. Hence we conducted tri-trophic food chain experiments using the algae *Rhodomonas salina* as the primary producer, the copepod *Acartia tonsa* as the primary consumer and the benthic life stage of the scyphozoans *Cyanea capillata* and *Chrysaora hysoscella* as secondary consumers. Two experiments were conducted examining the effects of different levels of CO_2 and food quality (Exp 1) and the effect of food quality and quantity (Exp 2) on the growth and respiration of scyphozoan polyps. Polyp growth and carbon content ($\mu g\ polyp^{-1}$) were not affected by the CO_2 treatments, but were significantly negatively affected by P-limitation of the food in *C. capillata* but not in *Ch. hysoscella*. Growth and carbon content were reduced in low food treatments but increased with decreasing P-limitation in high and low food treatments in *C. capillata*. Respiration was not significantly influenced by food quality and quantity in *C. capillata*. We conclude that phosphorus can be a limiting factor affecting the fitness of scyphopolyps and that P-limited food is of poor nutritional quality. Furthermore, OA, at least using realistic end-of-century scenarios, will have no direct effect on the growth of scyphistomae.

Introduction

One of the largely unresolved issues in the study of gelatinous zooplankton is the survival and growth of benthic stages of many scyphozoans. Not only is very little known about the exact locations of scyphozoan polyps in their natural habitats, we know even less regarding the feeding ecology of these polyps. The latter is mainly due to the almost complete lack of experimental work on this benthic life stage, with the notable exception of studies by Holst (Holst and Jarms 2007; Holst and Jarms 2010; Holst 2012b; Holst 2012a), Purcell et al. (Purcell et al. 1999; Purcell 2007; Bastian et al. 2011; Purcell et al. 2012) and Lucas (Lucas 2001; Lucas et al. 2012). These studies reported that polyps can be maintained and can strobilate when fed rotifers and/or *Artemia salina* nauplii. However, to our knowledge, no study has previously examined the effects of differences in both food quality and quantity on polyp growth dynamics. This is a large and important gap in our knowledge given the current debate on whether or not populations of gelatinous zooplankton are on the increase globally (Brotz et al. 2012; Condon et al. 2012), and what factors control the dynamics of gelatinous zooplankton. Indeed, many factors have been put forward to explain the (perceived) increase in jellyfish abundance in specific regions during the last decades: fisheries (Mills 2001); temperature increase (Holst 2012a; Lucas et al. 2012; Purcell et al. 2012; Webster and Lucas 2012); eutrophication (Purcell 2012; Purcell et al. 2013) and ocean acidification (Winans and Purcell 2010; Gattuso et al. 2013), as well as increased awareness and higher observational power (Brotz et al. 2012). We argue that it is difficult to make general statements on the factors driving population dynamics of these gelatinous zooplankters when basic knowledge of the polyp life-stage of scyphozoans is lacking.

Scyphomedusae compete with many fish species for food, and feed on their competitors' larvae and eggs (Barz and Hirche 2007; Sabates et al. 2010), but understanding jellyfish life cycle dynamics is not only important ecologically. Jellyfish blooms also affect aquaculture (Mills 2001; Purcell et al. 2007; Purcell 2012), fishing (Lynam et al. 2006), tourism and the operation of power plants (Purcell et al. 2007). It is important to note that the magnitude and extent of blooms of jellyfish with metagenic life cycles are directly related to population dynamics of benthic polyps (Boero et al. 2008; Holst 2012b) (Lucas et al. 2012). Furthermore, in contrast to the pelagic life stages, polyps can live for several years, and a single scyphopolyp is able to produce up to 4000 juvenile medusae (reviewed in Holst et al. 2007) during the temperature dependant strobilation process (Holst 2012a; Purcell et al. 2012). The survival of polyps is affected by an amalgam of biotic and abiotic factors. Examples include predation (Hoover et

al. 2012), thermal and salinity tolerance (Holst and Jarms 2010; Holst 2012a; Lucas et al. 2012) and food quantity (Purcell et al. 1999; Webster and Lucas 2012). However, no studies exist on the effects of food quality on survival and growth of polyps. This is an important gap in our knowledge, as we know that the composition and quality of prey at the base of marine food webs can change (Mackas et al. 2007; Ito et al. 2010), with consequences for upper trophic levels (Malzahn et al. 2007).

Changes in the nutrient concentrations in marine habitats stemming from eutrophication can influence not only the magnitude of productivity but also the composition of primary producer communities (Elser et al. 2007). Predators are affected by changes in the composition of their phytoplankton prey, as some phytoplankton are higher quality food than others (Irigoien et al. 2000; Malzahn and Boersma 2009). Moreover, changes in nutrient availability will also affect the nutrient composition of the phytoplankton and, as a result, can change the quality of the same species of phytoplankton as food for consumers (Sterner et al. 1998; Schoo et al. 2010). Importantly, these changes in body composition of organisms at the base of the food web can be transported to higher trophic levels where they impact consumer fitness (Malzahn et al. 2007; Boersma et al. 2008; Malzahn et al. 2010; Schoo et al. 2010). Thus, stoichiometric changes in key nutrients, especially in nitrogen (N) and phosphorus (P) relative to carbon (C), affect growth and productivity of phytoplankton, zooplankton and secondary consumers (Malzahn et al. 2010; Schoo et al. 2010). Nutrient stoichiometry of algae, i.e. the ratio between nutrients (N and P) and carbon can obviously be affected by both inputs of the macronutrients as well as by the availability of carbon. Currently, our world's oceans are receiving an enormous extra input of C due to increases in atmospheric CO_2. Atmospheric CO_2 will dissolve in sea-water and decrease pH, but it will also increase the availability of carbon for phytoplankton growth. Atmospheric CO_2 concentrations have already doubled from 200 ppm during pre-industrial times to currently close to 400ppm. Projections for further CO_2 increases vary, but all current scenarios agree that values around 1000 ppm are realistic by 2100 (Gattuso and Hansson 2011). Thus, marine consumers face both direct effects of decreased pH due to increased CO_2 as well as indirect effects as a result of higher carbon availability and changed stoichiometry of the food. Unfortunately, these direct and indirect effects are difficult to separate under natural settings. Since OA has been linked to changes in the frequency of gelatinous zooplankton (Attrill et al. 2007; Attrill and Edwards 2008; Haddock 2008) and only two studies, that we are aware of have reported the direct or indirect effects of OA on benthic polyp stages (Winans and Purcell 2010; Klein et al. 2014), it is of great interest to investigate whether a) OA affects benthic stages of scyphozoans, using realistic end-of-century scenarios, and b) whether a

potential effect of OA is mainly direct through changes in pH, or indirect through alterations of food quality. Benthic polyps of scyphozoans commonly occur in relatively shallow (1 to 15 m) waters (Toyokawa 2011). Although the pH of open ocean regions tends to be relatively stable, the pH of benthic areas of shallow, coastal waters often displays large fluctuations both seasonally and daily (Hofmann et al. 2011). Therefore, scyphozoan polyps have likely adapted to such large fluctuations and, thus, we hypothesise that scyphozoan polyps will be fairly insensitive to moderate decreases in pH. The present study examined the direct and indirect effects of pCO_2 / pH on polyps of *Cyanea capillata* and *Chrysaora hysoscella*, two jellyfish that often occur *en masse* during the summer months in the North Sea (Hay et al. 1990; Barz and Hirche 2007). Furthermore, we investigated how food quantity and quality interact, affecting the growth of *C. capillata* polyps. Specifically, the first experiment tested the hypothesis that pCO_2 / pH does not affect the growth dynamic of polyps of *C. capillata* and *Ch. hysoscella,* while a second experiment tested the null hypothesis that nutrient limitation in prey items, especially phosphorus limitation, and food quantity have no effects on the growth of polyps of *C. capillata*.

Materials and Methods

Two, tri-trophic experiments were conducted utilizing a primary producer, the cryptophyte alga *Rhodomonas salina*, a primary consumer, the calanoid copepod *Acartia tonsa* and two secondary consumers, polyps of the scyphozoan jellyfish *Cyanea capillata* and *Chrysaora hysoscella.* Phosphorus is the limiting nutrient for phytoplankton in many coastal seas (Elser et al. 2007), including the German Bight (van der Zee and Chou 2005), and thus we mainly focused on the effect of P-limitation. We cultured algae under different conditions, fed these algae to copepod nauplii and subsequently fed the nauplii to the polyps. First, we carried out an experiment with the two species of scyphozoans as top predators under two CO_2 conditions and two different food sources. Second, we focused on the more responsive species and investigated the interactions between food quality and quantity.

Primary producers

Stock cultures of the alga *(R. salina*) were cultivated in f/2-Medium with a nutrient concentration of 36.3 µmol L^{-1} NaH_2PO_4 and 882.5 mmol L^{-1} $NaNO_3$ (Guillard and Ryther 1962). We cultured the algae in sterile filtered (0.2 µm) seawater. For the algae used as food during the experiment, *R. salina* was cultured for four days in N- or P-limited media. The N

depleted (-N) medium was produced following Guillard and Ryther (1962) without the addition of nitrogen, consequently only the N contained in the filtered seawater was provided to the algae. The same protocol was followed for the P-depleted algae (-P), where no phosphorus was added. Differences between the nutrient composition of the different treatments are presented in figure 1. The experimental algae were cultured at 15°C and a 20:4 light:dark (L:D) cycle. Preliminary tests on algal growth rates under our laboratory conditions showed P and N limitation was reached after 4 days at around 0.9 x 10^6 cells mL^{-1} when inoculated with 0.3 x 10^6 cells mL^{-1}. Algal densities in the stock solutions were determined with a CASY cell counter (Schäfer System CASY Cell Counter and Analyser System). New cultures of *R. salina* were inoculated each day to guarantee constant food quality, and cultures were completely harvested every four days.

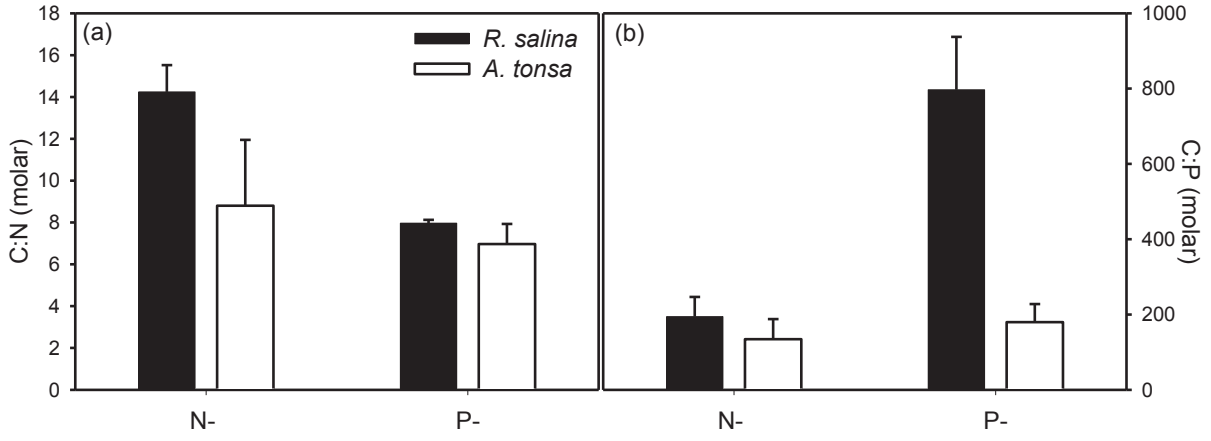

Figure 1: Stoichiometric measures of C:N (a) and C:P (b) (mean ± SE) for *R. salina* (black bars) and *A. tonsa* (open bars).

Primary consumers

Copepod eggs were obtained from a laboratory culture of the calanoid copepod *Acartia tonsa*. For the production of eggs, animals were kept in filtered natural seawater (salinity 31) in a 180-L tank in darkness at 18°C. The copepods were fed the alga *R. salina.* Eggs were collected daily and stored in airless falcon tubes at 4 °C until needed.

Before incubation, eggs were rinsed with fresh seawater and placed in 5-L plastic beakers at a density of 3000 individuals L^{-1}. Copepod nauplii were first fed 24 hours after hatching; 48 hours after incubation of the eggs. In order to avoid changes in the phosphorus content of the algae during incubation with the copepods, the eggs were incubated in phosphorus-free artificial seawater, adjusted to a salinity of 31 (salt: Tropic Marine). Algae were supplied to copepod nauplii with a minimum concentration of 10000 cells $individual^{-1}$ day^{-1}, which is considered to be *ad libitum* for larval stages (>1 mg C L^{-1}). The nauplii were fed the same amount of algal cells for each treatment to avoid food quantity effects. C:P and C:N ratio of the nauplii fed with nutrient limited algae used as food for the polyps are presented in figure 1. To guarantee a steady supply of food of constant quality for the polyps in the experiments, two new cultures of copepod nauplii were started each day.

The copepod nauplii grown on P-limited algae displayed a delayed development resulting in a time-lag of approximately one day compared to the copepod nauplii reared on N-limited algae. Therefore, P-limited nauplii were harvested eight days after hatching, when the majority had reached the sixth naupliar stage, and N-limited nauplii were one day younger. This ensured that copepod nauplii from different treatments were in the same developmental stage and had the same size. No differences in swimming or feeding behaviour were observed between the copepods fed different diets

Secondary consumer

Adult female medusae of the scyphozoan *Cyanea capillata* and *Chrysaora hysoscella* were collected around Helgoland and maintained in 5-L plastic aquaria filled with seawater at 18°C for 12 h. These medusae produced planula larvae, which were collected and transferred to crystallization dishes (8 cm x 4.5 cm, 150 mL volume) with filtered seawater. Since planulae colonize artificial substrates and favour attachment to the underside of these substrates (Brewer 1976; Holst and Jarms 2007), plastic petri dishes (35mm in diameter) were positioned on the water surface to provide floating settlement substrates. After a week at 15°C in darkness, a dense film of scyphozoan polyps had developed on the underside of the floating plastic petri

dishes. During the following three weeks, the young polyps were fed mashed, freshly hatched nauplii of the brine shrimp, *Artemia franciscana*. Polyps were fed every other day for a 3-h period by adding 7.5 mL of *Artemia*-solution to each container. Petri dishes were subsequently transferred to containers with fresh seawater. After four weeks, when most polyps had reached the 8 tentacle stage, they were fed living *Artemia* nauplii which were added to the containers in excess every second day with a subsequent water change after 3 h. During cultivation and experiments, prey ingestion by individuals in all treatments (including those provided N- and P-limited prey) was confirmed by observing the colour of the polyps. Polyps are translucent and ingested prey (*Artemia franciscana* and *A. tonsa*) were clearly visible. Polyps were used for experiments after reaching approximately 0.5 mm in diameter. Petri dishes with 10 attached polyps were used as experimental units (UEs) for each replicate in each treatment.

Experiment 1: Effects of CO_2 and food quality

During the experiment, *C. capillata* and *Ch. hysoscella* polyps were cultured in P- and N-free artificial seawater to exclude potential dissolved nutrient intake over the polyps' body surface. Artificial seawater for water exchange was stored in plastic tanks (60 L) and every single UE was constantly aerated in a 1-L beaker through glass pipes with two different CO_2 concentrations, 200 and 800 ppm. The UEs were covered with plastic wrap and elastic bands to reduce gas exchange with the atmosphere and to be in equilibrium with the gas constantly bubbling through the water. Total alkalinity and pH of the seawater used for the experiment were measured once during the experiment. Plastic petri dishes were always floating on the water surface. Polyps of *C. capillata* and *Ch. hysoscella* were fed either 100 % N- or 100 % P-limited copepod nauplii, offered at 4 nauplii mL^{-1} (400 nauplii EU^{-1} day^{-1}) in each treatment every other day for 3 h followed by a water exchange. These two treatments were crossed for each species with CO_2 concentration (200 and 800 ppm) in a full factorial design, resulting in 4 treatments for each species, and 7 replicates in each treatment. The duration of the experiment was 14 days. Three polyps of each UE were marked with a water resistant pen on the surface of the petri dishes, and polyp diameters were measured individually at the beginning of the experiment and thereafter every five days. After 14 days the animals were harvested and the carbon content of the polyps was established.

Experiment 2: Food quality and quantity

Based on results of the CO_2 experiments, only *C. capillata* polyps were chosen for the experiment on the effects of food quality and quantity. Polyps had the same preconditioning as described for the CO_2 experiment. During the experiment, *C. capillata* polyps were kept in crystallization dishes (8 cm x 4.5 cm, 150 mL volume) filled with 100 mL artificial seawater and fed along a nitrogen-to-phosphorus limited gradient every other day for 3 h followed by a water exchange. The copepod nauplii from P- and N-limited cultures were mixed prior to being fed to polyps, resulting in five quality treatments: 1) 100 % N-limited nauplii; 2) 75 % N-limited and 25 % P-limited nauplii; 3) 50 % N-limited and 50 % P-limited; 4) 25 % N-limited and 75 % P-limited nauplii; 5) 100 % P-limited nauplii. These five treatments were offered to polyps at either 'low' or 'high' quantities of 0.4 and 4 nauplii mL^{-1} day^{-1} (40 or 400 nauplii UE^{-1} day^{-1}), respectively, altogether resulting in 10 treatments. The lower amount is equivalent to the average of the densities of nauplii at Helgoland Roads (54° 11' N, 7° 53' E)(Meunier et al. 2015), whereas in the high prey quantity treatment, prey nauplii were always present in all experimental units after 24 hours of feeding, no nauplii were left in the low food quantity treatments. The treatments were randomly assigned to each of 100 UE, resulting in 10 replicates for each of the treatments. The experiment was conducted for 26 days. The diameter of three polyps on every UE was measured at the start of the experiment and then measured on the same individuals at weekly intervals. After 26 days the polyps were removed and analysed for carbon content. Furthermore, we also measured respiration rates of the polyps after the experimental period to establish whether food quality or food quantity influenced rates of respiration.

Analytical procedures

Due to the fact that gelatinous organisms have no exo- or endoskeleton or other stabilisation structures, it is difficult to make accurate measurements of the body size, especially of scyphistomae, that prefer settling upside down. Therefore, we tested a new method to carry out *in vivo* size measurements of polyps. We poked the polyps several times with a blunt curved needle to induce their maximum contraction. Images of the oral side of the contracted polyps were taken by a binocular (Olympus; SZ18) equipped with a camera system and the longest total body diameter was measured using the Software CellSens Dimension, Version 1.6. However, most polyps contracted to an almost perfect circle, which means the longest distance resembles the diameter of the contracted polyp. The diameter data were compared to the carbon content data of the polyps measured at the end of the experiment.

To establish carbon content measurements all ten polyps were stripped off the plastic petri dish undersides with a needle, rinsed in distilled water, collected in tin-cups and freeze dried before analysing. For the analysis of carbon content of the algae, approximately 4 x 10^6 cells were filtered onto pre-combusted and washed Whatman GF/F filters. For the analysis of copepod carbon, 100 individuals were filtered onto filters and packed into tin foil. The carbon content of the samples was measured with a CHN analyser (elementar, vario MICRO CUBE).

Respiration measurements were carried out at the end of the second experiment. To account for differences in the final size of polyps in different treatments at the end of the experiment, dry weight-specific rates of respiration (ng O_2 µg $polyp^{-1}$ h^{-1}) were calculated and compared at each of the five food quality treatments (n = 6 replicate measurements) and two feeding levels (high and low). Measurements were made at 15°C using a Unisense A/S Micro-respiration System (Unisense, Aarhus, DK). The system allows repeated O_2 measurements to be made on organisms within 750 µL chambers. Each chamber was equipped with a stainless steel mesh that separated a stir magnet (120 rpm) from polyps. Polyps were measured in small groups (6 to 10 individuals $chamber^{-1}$) over the course of 4 to 5 hours. Measurements were performed in runs. Each run contained 8 chambers with polyps and one chamber without polyps (blank). Treatments were measured randomly with respect to run. After loading the polyps into chambers, a short (10 to 20 min) acclimation period was provided before measurements of O_2 concentration commenced. In chambers with polyps, O_2 concentrations never decreased below 55 % saturation. Respiration rates (of chambers with polyps and blanks) were calculated from the linear decrease in O_2 concentration versus time. The respiration rate of the blank chamber was subtracted from that of chambers with polyps (rates in blanks were always < 20 % those of polyps). Gambill and Peck (2014) provide a detailed description of the methods used to make the respiration measurements. After the measurements, polyps were washed in distilled water to eliminate remaining salt, transferred into tin cups and then were freeze-dried, weighed and used for carbon measurements.

pH and total alkalinity (TA)

The target CO_2 concentrations used in the experiments were established by bubbling a CO_2 / air mixture into seawater. The CO_2 outflow was continuously controlled (HTC-Hamburg, model: MK 3-2-s). The CO_2 concentration was checked by analysing pH and total alkalinity (TA) of seawater in tanks that were preconditioned for water exchange for 48 h. This CO_2 manipulation is standard procedure and several pre-experiments have confirmed the constant conditions in the experiment. The pH was measured with a ProLab 3000 pH meter with an

IoLine pH combination electrode with temperature sensor (type IL-pHT-A170MF-DIN-N). The pH and TA were measured at 15°C. 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). Measurements were corrected using Certified Reference Material (CRM, Batch No. 104, Scripps Institution of Oceanography, USA). Calculations of carbon dioxide partial pressure were made with the program CO2Calc (Robbins et al. 2010), using the dissociation constant of carbonic acid of Mehrbach et al. (1973), as modified by Dickson and Millero (1987), and dissociation constants for H_2SO_4 from Dickson (1990).

Statistical analysis

Growth rates of the polyps were calculated by using the equation $gr = (\ln B_t - \ln B_0) / \Delta t$, where B_0 and B_t are the initial biomass (C content, µg C $polyp^{-1}$) and the biomass after 26 days, respectively, and the experimental duration (Δt, day). The relationship between carbon content and size in diameter, and carbon content and growth rate at the end of the experiment was assessed by linear regression analysis using SigmaPlot 11.2.0.5 (Systat Software, Inc.). Analysis of the diameter change of the polyps over the experiment duration was calculated with a Repeated Measures (RM) ANOVA followed by a Tukeys HSD post hoc test using Statistica 9.1 (StatSoft. Inc.). A one-way ANOVA test for differences in weight-specific respiration rates was conducted with R 2.15.2 (R Core Team 2012). Polyps used as control (starving) during the experiment decreased in diameter and were not included for any statistical analysis. All data were tested for normality and equal variance before statistical tests; no transformations were necessary.

Results

Testing the suitability of the poking method for the analysis of growth rates, we observed a highly significant ($P < 0.001$) linear regression between the natural logarithm (ln) of the diameter and the ln of the carbon content with a positive slope of 2.3 and $r = 0.916$. Thus, repeated measurements of live polyps can yield accurate estimates of biomass (Fig. 2), and can be used to estimate growth.

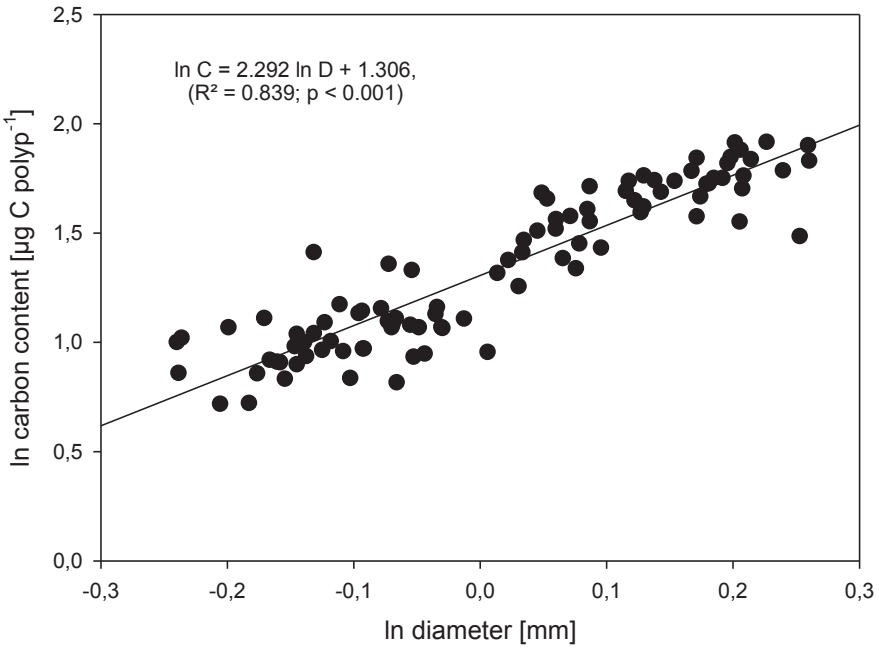

Figure 2: Linear regression of natural logarithm (ln) of carbon content (C, µg C polyp^{-1}) and ln of diameter (D), mm) of polyps of *C. capillata*. (n = 95)

CO_2 experiment

The final diameter and weight of polyps of both *C. capillata* and *Ch. hysoscella,* reared within 200 and 800 ppm CO_2 were not significantly different despite a difference in pH between the treatments of 0.5 (8.4 for 200 ppm and 7.9 for 800 ppm CO_2). The response to the different foods differed between the two species. Polyps of *C. capillata* fed N-limited copepod nauplii reached a mean diameter of 1.17 mm regardless of CO_2 concentration, and 0.9 mm for polyps fed with P-limited nauplii (Fig. 3a).

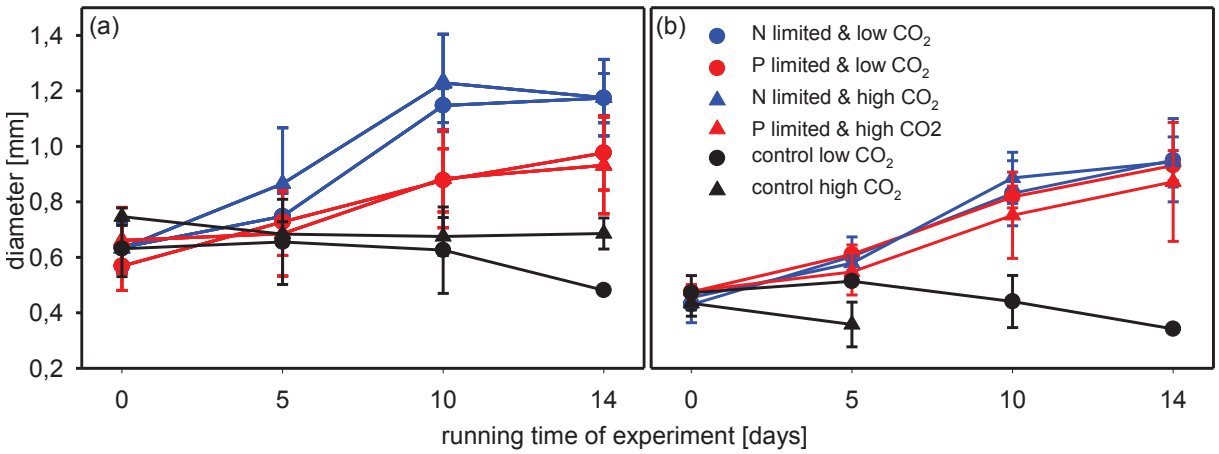

Figure 3: Development of *C. capillata* (a) and *Ch. hysoscella* (b) polyps as diameter (mean ± SD, mm) over time (days) fed on either N limited (blue coloured) or P limited (red coloured) copepod nauplii and kept under either low CO_2 (200ppm, circle) or high CO_2 (800 ppm, triangle) condition. Control treatments (low CO_2 = black circle; high CO_2 = black triangle) were polyps under starvation. (n = 2) *Ch. hysoscella* control high CO_2 polyps died between the 5th and 10th day of the experiment. *Error bars*: standard error

Independent of the CO_2 concentration, there was also a significant effect of food quality on the carbon content of *C. capillata* polyps (Table 1: two-way ANOVA, $P < 0.001$). After 14 days, the C content of polyps fed P-limited copepod nauplii was 11.7 µg C polyp^{-1} which was one third of that (34.1 µg C polyp^{-1}) of individuals fed N-limited nauplii (Fig 4a). In contrast, the growth in length and biomass of polyps of *Ch. hysoscella* was not significantly different between the two CO_2 treatments or food quality treatments (Fig. 3b, Fig 4, Table 1)

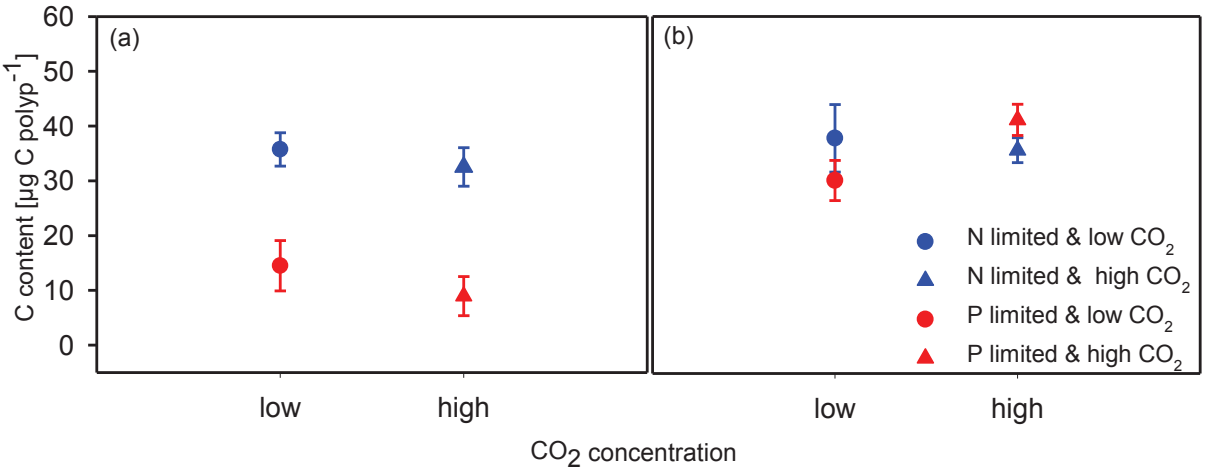

Figure 4: Carbon content (mean, µg C polyp^{-1}) at low and high CO_2 concentration for N limited (blue) and P limited (red) treatments for *C. capillata* (a) and *Ch. hysoscella* (b). (n = 10) *Error bars*: standard error.

Table 1: Results of two-way ANOVA for polyps of *Cyanea capillata* and *Chrysaora hysoscella* at the end of the experiment for the OA experiment using CO_2 and quality as independent factors and carbon content (µg C polyp^{-1}) as dependent variable.

	Factors & Interactions	df	MS	F	p
C. capillata	**CO_2**	1	0.008	0.390	0.539
	Quality	1	0.294	15.265	< 0.001
	CO_2 x Quality	1	0.001	0.048	0.829
	Error	23	0.019		
Ch. hysoscella	**CO_2**	1	0.001	0.049	0.826
	Quality	1	0.000	0.021	0.886
	CO_2 x Quality	1	0.000	0.008	0.928
	Error	21	0.014		

Quality and Quantity experiment

In this experiment, the diameter of the same polyp was repeatedly measured and the mean increase in diameter was significantly greater in the high (0.8 mm) versus low (0.2 mm) food quantity treatments (RM ANOVA, $P < 0.001$, Table 2, Fig. 5 a & b). Furthermore, the quality of the food had a significant effect on polyp growth regardless of the quantity of food (RM ANOVA, $P < 0.05$, Table 2).

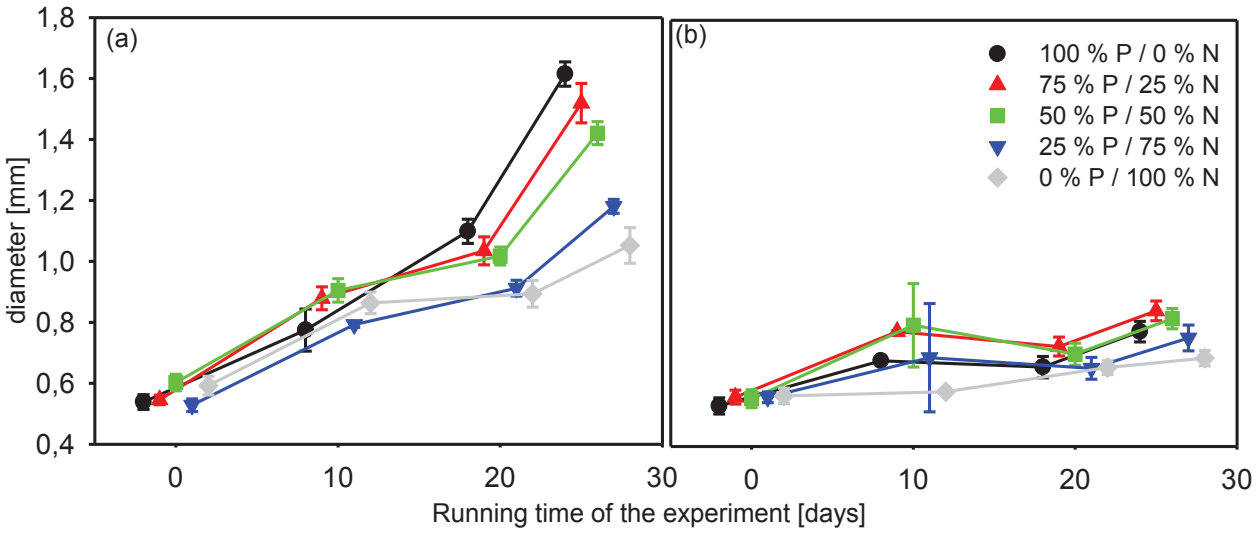

Figure 5: Development of *C. capillata* polyps as diameter (mean ± SE, mm, n = 3) over time (days) fed on copepod nauplii along a phosphorus gradient for high quantity treatment (a) and low quantity treatment (b). Error bars may disappear, as standard error is too small. Treatments were shifted along x-axis for each sampling day for better visualisation. 100 % P = no P limited copepod nauplii were used for polyp feeding, 75 % P = 25 % of the copepod nauplii were P limited that were used for feeding, 50 % P = 50 % of the copepod nauplii were P limited that were used for feeding, 25 % P = 75 % of the copepod nauplii were P limited that were used for feeding, 0 % P = copepod nauplii used for feeding polyps were all P limited.

Table 2 Results of two-way RM ANOVA for polyps of *Cyanea capillata* using quantity and quality over time as independent factors and diameter (mm) as dependent variable.

Factors & Interactions	df	MS	F	p
Quantity	1	1.801	47.388	< 0.001
Quality	4	0.132	3.463	< 0.05
Quantity x Quality	4	0.023	0.599	0.669
Time	3	1.199	222.755	< 0.001
Time x Quantity	3	0.333	61.790	< 0.001
Time x Quality	12	0.033	6.082	< 0.001
Time x Quantity x Quality	12	0.021	3.858	< 0.001
Error	45	0.005		

For the high quantity treatment, the total carbon content of *C. capillata* polyps increased with increasing phosphorus availability in the copepod nauplii (Fig. 6a). The C content of polyps fed with P-depleted food was one third that of polyps fed with 100% P-replete food. The highest polyp carbon content was observed when polyps were fed high amounts of food without any P limitation, even though the prey was composed of 100 % N limited nauplii (Fig. 6a; Linear regression, $c = 0.4\ q + 26.757$; $r^2 = 0.68$; $P < 0.001$). The low quantity treatment also showed significant reactions to different P contents in offered food after 26 days (Fig. 6a; Linear regression, $gr = 0.0343\ q + 8.729$, $r^2 = 0.199$, $P = 0.002$), albeit with a slope that was less steep. The linear regression of the growth rate (day^{-1}) over the proportion of phosphorus replete copepod nauplii in the diet showed a significant positive slope (Fig. 6b). *C. capillata* polyps fed high quantity were growing faster than polyps fed with low food concentrations. The high quantity treatment polyps were more affected by phosphorus concentration in their food and showed the most rapid growth rate when P was offered at 100 % (Fig. 6b; Linear regression, $gr = 0.000196\ q + 0.0238$; $r^2 = 0.654$; $P < 0.001$). Polyps offered low prey concentration grew more slowly than those offered high concentrations of prey, but also displayed a significant increase in growth rate with increasing phosphorus concentration in the offered nauplii (Fig. 6b; Linear regression, $gr = 0.0000736\ q + 0.00916$; $r^2 = 0.222$; $P < 0.001$).

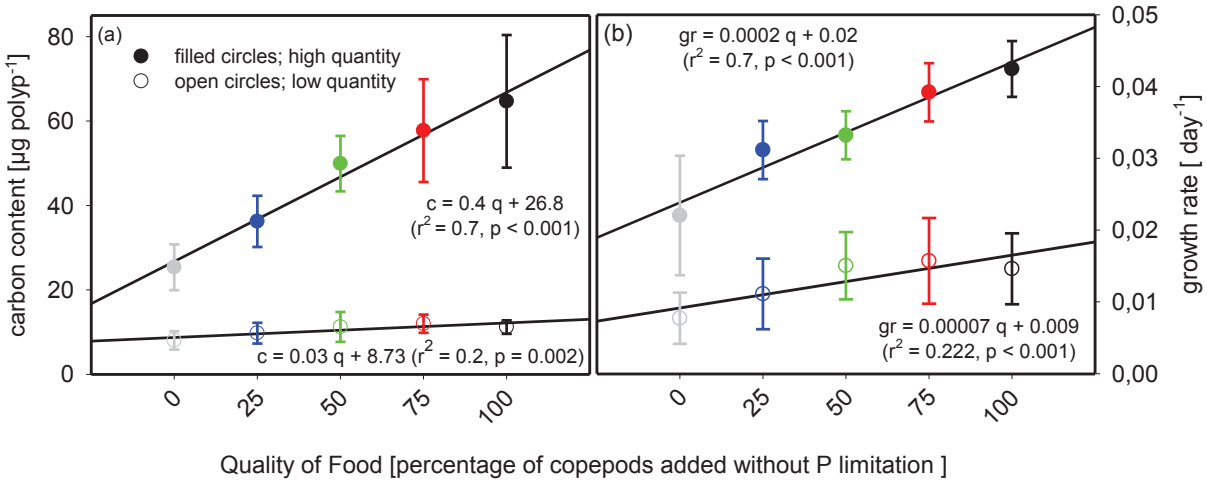

Figure 6: Reactions of *C. capillata* polyps to different P content. Regression lines indicate significant relationships between the percentage of P offered with copepod nauplii as food and carbon content (a) or growth rate (b) over five different food qualities at the end of the experiment, i.e. after 26 days. **a** carbon content (µg Polyp^{-1}; n = 50); **b** growth rate (day^{-1}; n = 50); c = carbon content, gr = growth rate, q = P content; *Error bars:* standard error.

Polyps in the high prey quantity treatment had significantly higher weight-specific respiration rates than polyps in low quantity polyps (ANOVA, $F_{1,45} = 24.33$, $P < 0.001$). The mean (± SE) weight-specific respiration rate of high quantity polyps (across food quality treatments) was 0.681 (± 0.042) ng O_2 µg polyp^{-1} h^{-1}, which was 2- times higher than that (0.343 (± 0.052) ng O_2 µg polyp^{-1} h^{-1}) for low quantity polyps. The mean DW of polyps in the high and low quantity treatments was 140 and 62 µg, respectively, thus total (polyp^{-1}) respiration rates were much higher in the former than the latter. In terms of prey quality, polyps in poor food quality treatments (0 and 25% P) had similar weight-specific rates of respiration as polyps in high food quality treatments (75 and 100% P) for both levels of prey quantity (Fig. 7).

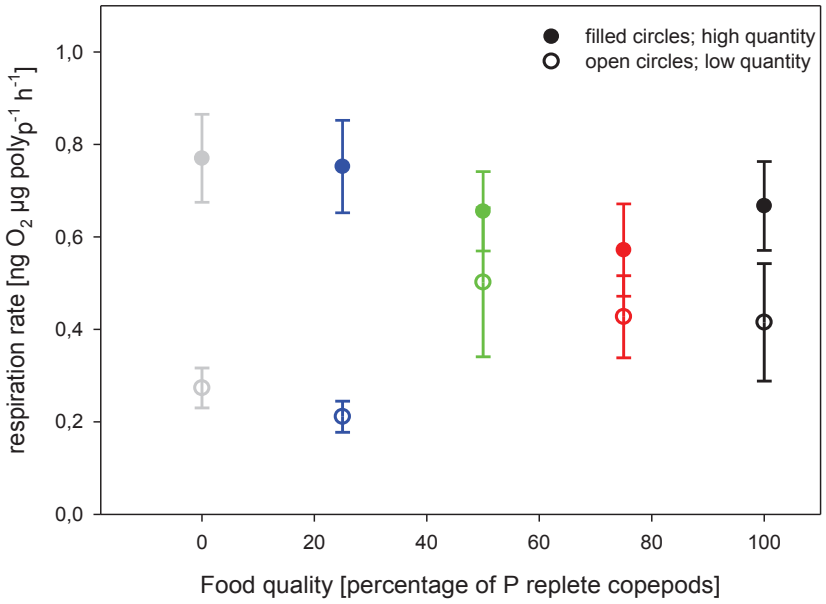

Figure 7: Weight-specific respiration rate (ng O_2 µg $polyp^{-1}$ h^{-1}) of *C. capillata* polyps maintained at different food qualities (percentage of P replete copepod nauplii) for high (filled circles, n = 6) and low quantity (circles, n = 6) treatment. rr = respiration rate, q = quality of food. *Error bars*: standard error.

Discussion

In this study, we have shown that the growth rate of polyps of *Cyanea capillata* and *Chrysaora hysoscella* were not affected by the CO_2 concentration in the water. The scyphozoan *Cyanea capillata* was affected by both the quantity and quality of the food. This is in accordance with the growing body of evidence that food quality effects can travel up the food chain, and that nutrient limitations in algae can even affect secondary producers (Boersma et al. 2008). In contrast, growth rates of *Chrysaora hysoscella* polyps were not affected by the quality of the food.

Effects of Ocean Acidification

To our knowledge, two studies have examined the effects of OA on gelatinous zooplankton in the field. Attril et al. (2007) and Richardson and Gibbons (2008) both analysed field data and

correlated the abundance of gelatinous zooplankton with seawater pH, but only the former study reported a correlation between field abundance of jellyfish and in situ pH; it is important to note that these are correlative studies and no direct evidence exists that pH is causally linked to the abundance of gelatinous zooplankton. An additional two laboratory studies investigated the effects of pH on jellyfish, demonstrating a high tolerance of polyps to reduced pH (Winans and Purcell 2010; Klein et al. 2014). Similarly, our study showed that polyps of *C. capillata* and *Ch. hysoscella* were not influenced by direct effects of OA, thus confirming the results of previous work. However, the results of the present study suggest that polyp growth may be impacted by OA indirectly, through changes in food quality as discussed in the following sections.

Effects of changes in food quality (nutrient stoichiometry)

Our results show that P-limitation affects the growth of *C. capillata* polyps. Interestingly, no effects of food quality were found for *Ch. hysoscella.* A potential reason for this could be that we only contrasted two limited (N- and P-) food sources, and did not include copepods grown on full-medium algae as food. Theoretically, growth of *Ch. hysoscella* could have been highest on the non-limited food chain, with animals being N-limited in one case and P-limited in the other one. However, this is not very probable, because the difference in N-content between nitrogen limited and non-limited copepods is small (Malzahn et al. 2007), much smaller than the differences in P-contents between phosphorus limited and non-limited copepods. Hence the likelihood of actual N-limitation is low, especially since typical C:N ratios of gelatinous zooplankton are in the order of 4-5 (Kogovsek et al. 2014), fairly close to the C:N ratios of the copepods in our study. Thus, we cannot explain the difference between the species. Previous research by Malzahn et al. (2010) showed that the Limnomedusa *Gonionemus vertens* was not affected by the nutrient content of its copepod food, whereas Schoo et al. (2010) reported a negative effect of prey with high P-content on the growth of the ctenophore *Pleurobrachia pileus*. The growth rate hypothesis (Elser et al. 1996) links phosphorus demand of organisms with their growth rate, stating that higher growth is only possible with high amounts of nucleic acids which are rich in phosphorus. Thus, it could be that the difference in vulnerability to P-limitation is linked to the maximum growth rates for the different species. Unfortunately, no growth rates are available for the studies of Malzahn et al. (2010) and Schoo et al. (2010), as in

both cases the experiments were carried out with adult individuals, so we cannot test whether the species vary widely in growth rate. However, in our study *C. capillata* had a higher growth rate than *Ch. hysoscella*, which would be consistent with the growth rate hypothesis. Further research investigating a whole suite of different jellyfish species is necessary to be able to judge how we can generalize the results obtained here.

Prey nutrient stoichiometry has been previously shown to influence the growth of several primary and secondary consumers including protist grazers (Nakano 1994; Malzahn et al. 2010), crustaceans such as *Daphnia* (Ryther 1954; Urabe and Watanabe 1992; Boersma 2000), copepods (Malzahn et al. 2010; Malzahn and Boersma 2012), European lobster (Schoo et al. 2012) and fish (Hood et al. 2005; Malzahn et al. 2007). In this regard, polyps of *C. capillata* behave just like other animals: P is an essential element fuelling their growth. It remains to be seen whether this also is the case for other stages of the metagenic life cycle. One explanation for the fact that the studies of Malzahn et al. (2010) and Schoo et al. (2012) and this study found different results could also be the differences in the life stages investigated.

For *C. capillata*, we demonstrated a significant effect of food quality on polyp growth at both high food quantity as well as low food quantity. This result is in line with the findings of Boersma and Kreutzer (2002) and Schoo et al. (2012). Those authors argued that, even at low prey availability and resulting very low growth rates, processes such as cell regeneration take place and need to be supplied with building blocks such as phosphorus and not just with energy. This contradicts the observations by Sterner and Robinson (1994) who stated that, at low food availability, individuals only incur costs of maintenance metabolism, anabolic processes are not of importance and, thus, substances such as nitrogen and phosphorus to build tissue are not needed. Here we show that this is not the case.

Pitt et al. (2013) concluded that beside the relatively large size, low body carbon content and simple body plan of medusae, complex metagenic life histories strategies, including benthic polyp stages, could be a reason for surviving and even developing blooms in habitats with nutritional conditions that are unacceptable for non-gelatinous organisms. One unexpected result is our observation that food quality did not affect respiration rates of the polyps. Based on the literature (Darchambeau et al. 2003; Hessen et al. 2004; Malzahn et al. 2010) we expected that respiration rates would increase with increasing C:P of the food. Animals receiving severely unbalanced food have an excess of carbon, which they need to eliminate to maintain their nutrient ratio homeostasis. Several pathways exist to do so, the most important of which are excretion and respiration (Pitt et al. 2009; Condon et al. 2011; Pitt et al. 2013; Schoo et al.

2013) and especially for jellyfish, production of mucus (Condon et al. 2011). The respiration rates were highest at the lowest P-food at least for the high quantity treatments but, due to the relatively high variation, these results were not significant. Thus, we propose that, similar to adult copepods (Schoo et al. 2013), excretion and not respiration is the more important channel to eliminate excess carbon in polyps of *C. capillata*. More research is necessary to show whether this indeed is the case.

Our results demonstrate polyp growth in *C. capillata* was possible when food quality was low even if the quantity was very limited. However, growth was reduced in P-limited conditions. Polyp growth is crucial for the development of jellyfish blooms, since the number of ephyrae produced by the polyp increases with the polyp's size (Russel 1970; Lucas et al. 2012). Previous studies have demonstrated the high tolerance of scyphozoan polyps to extreme environmental conditions such as low salinities (Holst and Jarms 2010) and hypoxic conditions (Condon et al. 2001) and the present results indicate that even nutrient limitation can be tolerated to a high degree. Moreover, polyps of many scyphozoan species produce chitin-covered cysts as reserves of organic compounds enabling them to survive periods of low food availability and predation (Olariaga et al. 2014). We suggest that the high tolerance of the scyphozoan polyp generation to changing environmental conditions in combination with their various strategies of asexual reproduction and cyst formation is the basis for the repeated successful recruitment of scyphomedusae and the development of jellyfish blooms.

Implications

How do our results help our understanding of jellyfish bloom formation? We have learned that direct effects of OA on gelatinous zooplankton are not likely to be large, at least not with realistic levels of OA projected at the end of this century. As early life stages of fish may be more vulnerable to both direct (Baumann et al. 2012; Frommel et al. 2012; Frommel et al. 2014; Murray et al. 2014) as well as indirect (food quality) (Malzahn et al. 2007) effects of increased CO_2 levels, ongoing OA may indirectly favour gelatinous zooplankton. However, indirect effects of OA, through changes in the quality of the food (Urabe et al. 2003; Rossoll et al. 2012; Schoo et al. 2013; Verschoor et al. 2013), exacerbated by decreasing nutrient inputs, may be important to both jellyfish (this study) as well as fish (Malzahn et al. 2007). Future research will need to focus on both direct and indirect mechanisms to understand how climate change and other pressures will affect jellyfish bloom dynamics.

Chapter II

Ocean acidification and temperature affect polyps and ephyrae of the Scyphozoans *Chrysaora hysoscella* and *Cyanea capillata*

Abstract

Increasing atmospheric CO2 concentration not only contributes significantly to global warming but also increases CO2 concentrations in the world's oceans, known as ocean acidification (OA). Both increasing temperature and OA affect marine ecosystems. In most studies, effects of OA and temperature are investigated separately. However, the negative effects on different marine species are already known, but there is less information about the interactive effects of warming and OA. Gelatinous zooplankton and their juvenile stages especially are poorly studied in this framework. We have tested the reaction of Chrysaora hysoscella polyps and Cyanea capillata ephyrae to increasing temperature and OA using a temperature table at a gradient from 10 °C to 31 °C for polyps and 4.4 °C to 25.5 °C for ephyrae at two different CO2 concentrations (200 ppm and 800 ppm for polyps, 1000 ppm for ephyrae). Growth and carbon content of polyps and ephyrae were significantly affected by temperature but not affected either by the CO2 concentration or by an interaction of both factors. We conclude that increasing seawater temperature as part of the global warming will affect Ch. hysoscella polyps and C. capillata ephyrae and that CO2 concentrations predicted for the end of the 21st century will not affect the development of the investigated juvenile stages.

Introduction

The earth is warming at an alarming rate. This warming is mainly caused by the increase of CO_2 and other greenhouse gasses in the atmosphere, a process that is likely to continue in the coming decades, even if we reach the targets set by various governments in the COP21 agreement. Currently, the pCO_2 level in the atmosphere is around 400 ppm (Le Quere et al. 2009), a value much higher than the pre-industrial concentrations of 280 ppm. Predictions in the last IPCC report for end of the twenty-first century atmospheric CO_2 concentrations range from 700-1000 ppm (IPCC 2014), depending on the scenario. At the same time as CO_2 is warming the earth's atmosphere, it dissolves in water, leading to acidification of marine environments through an increase in proton concentration in the water column. This ocean acidification (OA) can have strong effects on sensitive marine organisms, specifically those that rely on calcification (Melzner et al. 2009; Jansson et al. 2015; Queiros et al. 2015; Steckbauer et al. 2015). Despite the fact that warming and acidification are concurrent processes, research studies often focus on the effects of single stressors on individual species or ecosystems alone, instead of investigating synergetic effects. In those studies that included multiple stressors, interactions between different stressors have frequently been observed (Passow and Laws 2015; Przeslawski et al. 2015; Algueró-Muniz et al. 2016; Malzahn et al. 2016). Ocean acidification influences critical ecological processes such as respiration, photosynthesis and nutrient dynamics, which are important drivers of calcification, ecosystem structure, biodiversity, and, ultimately, the health of the ecosystem (Guinotte and Fabry 2008). However, our knowledge of how changing temperatures interact with OA is limited.

Homeothermic organisms tolerate a very limited range of internal temperatures, since molecular, cellular and systemic processes are adapted to a constant body temperature. In contrast, the body temperature of poikilotherms correspond to environmental temperatures and thus, their 'internal machinery' must be able to deal with a wide temperature range (Pörtner and Farrell 2008). Each organism has a species-specific temperature range limiting survival, growth and reproduction. Pörtner (2001) formulated a theoretical framework concerning the capacity of organisms to deal with different temperatures (Fig 1), a concept called OCLTT (oxygen and capacity limited thermal tolerance). In short, every organism has a thermal tolerance curve with a maximum in the response variable (growth, reproduction) representing the optimal performance temperature (T_{opt}). Based on the tolerance curve, both flanks represent first lines of limitation in thermal tolerance. These earliest, ecologically relevant thermal tolerance limits are called pejus temperature (T_{pej}) (Schiffer et al. 2014). Outside this temperature, organisms'

functionality and performance is affected. Further outside the optimal temperature range the critical temperature (T_{crit}) is reached, beyond which survival is time-limited, as only a passive anaerobic existence is possible (Frederich and Pörtner 2000). The thermal tolerance curve can vary in width dependent on the species, i.e. stenoecious species have a narrower window than euryoecious ones. Pörtner and Farrell (2008) also pose that other stressors affect the thermal tolerance window, essentially lowering the performance at all temperatures, which results in pejus and critical temperatures closer to the optimal temperature (Fig 1). Hence, one would expect that under additional OA stress the thermal window of many species should become smaller.

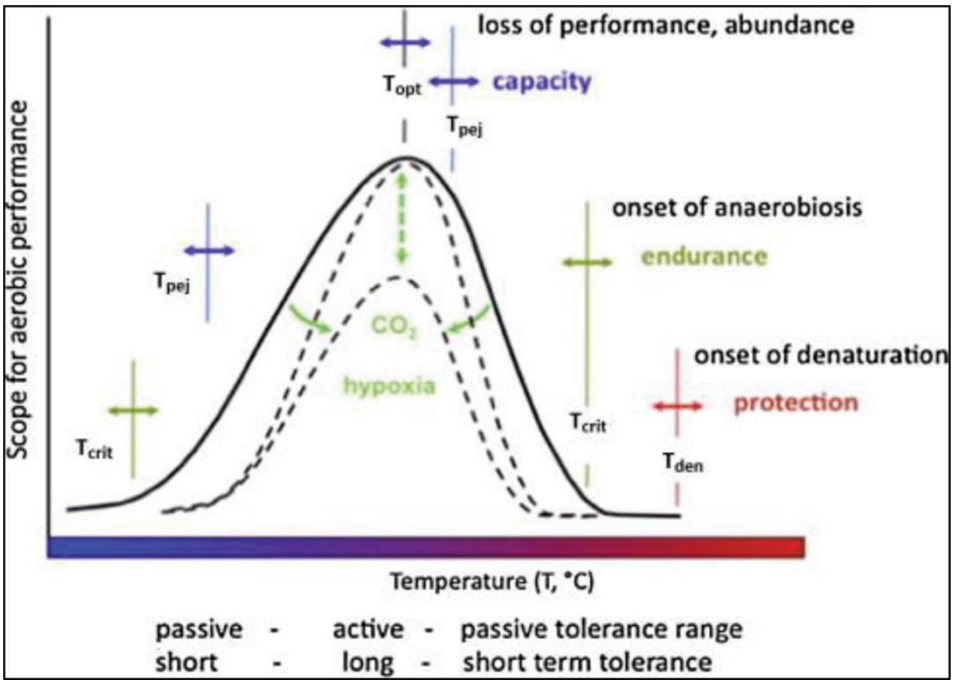

Figure 1: The concept of oxygen and capacity limited thermal tolerance (OCLTT) providing an explanation for the specialisation of animals on specific, limited temperature ranges and their sensitivity to temperature extremes. T_{crit} = Critical temperature where there is an onset of anaerobic metabolism; T_{pej} = Pejus temperature above and below which oxygen supplies to tissues become sub-optimal; T_{opt} = Optimal temperature; T_{den} = Denaturation temperature where molecules lose integrity (Bijma et al. 2013).

There is currently considerable discussion in the literature as to whether populations of gelatinous zooplankton are increasing globally or not (Brotz et al. 2012; Condon et al. 2013). In fact, reports exist that link increases in population densities to increases in temperature (Bamstedt et al. 1999; Vansteenbrugge et al. 2015) or decreases in pH. However, there is no work that combines different stressors, with the exception of the recent study by Algueró-Muñiz et al. (2016) that considered only two temperatures. Thus, the available data are not sufficient

for unambiguous conclusions on the factors controlling gelatinous zooplankton dynamics. One reason that this task is so challenging is the complex life-cycle of many species. Scyphozoans usually have metagenetic life cycles comprising a sessile asexual reproductive polyp generation and a planktonic sexual reproductive medusa generation. The juvenile medusae (ephyrae) are released by the polyps in an asexual reproduction process (strobilation) but data concerning the ecology and survival of juvenile medusa stages are rather scarce. Previous studies have shown that polyps are highly tolerant to changes in abiotic environmental conditions, for example low oxygen concentrations (Condon et al. 2001; Gambill and Peck 2014) and variances in the nutrient composition of food (Lesniowski et al. 2015). Scyphozoan polyps are perennials and in temperate waters they may be exposed to wide-ranging and fluctuating biotic and abiotic environmental variables including temperature and pH (Lucas et al. 2012). In comparison to polyps, ephyrae are probably less robust (Bamstedt et al. 1999). Therefore, we would expect a wider thermal window for polyps in comparison to ephyrae. Laboratory experiments have indicated that the strobilation of North Sea scyphozoans is usually triggered by a temperature decrease and ephyrae are often released early in the year, when water temperatures are still low (Holst 2012a), so we would expect a narrow thermal window for ephyrae, with a negative effect of temperatures much warmer than usual North Sea winter temperatures. Furthermore, based on the OCLTT-Model, we would expect a narrowing of the thermal window with increasing CO_2 for both early life stages, polyps and ephyrae.

For our study, we chose two scyphozoan species, *Chrysaora hysoscella* (Linnaeus, 1767) and *Cyanea capillata* (Linnaeus, 1758). The adult medusae of both species regularly appear in the German Bight, in the North Sea, in the summer months (Russel 1970; Hay et al. 1990; Barz and Hirche 2007; Doyle et al. 2007). Mature *Ch. hysoscella* medusae are distributed mainly in the southern North and Irish Seas (Russel 1970; Hay et al. 1990; Doyle et al. 2007). However, the distribution and temperature tolerance of *Ch. hysoscella* polyps are unknown since they have never been documented from the field in the North Sea and laboratory investigations into this species are limited (Holst 2012a). Therefore, we designed laboratory experiments to investigate combined temperature and CO_2 effects on the polyp stage of *Ch. hysoscella* in order to draw conclusions about the potential effects of temperature increase and OA on their future occurrence in the North Sea.

In contrast to *Ch. hysoscella*, *C. capillata* medusae are rare in the southern part of the North Sea and Irish Sea (Hay et al. 1990; Doyle et al. 2007). A few records of *C. capillata* polyps in the field revealed strobilation periods from March to June in the Gullmar Fjord (West coast of

Sweden) (Gröndahl and Hernroth 1987) and in winter at Helgoland (German Bight) (Hartlaub 1894). These previous results indicate, that *C. capillata* ephyrae are probably adapted to colder temperatures and may suffer in warmer temperatures. Our laboratory experiments were conducted to verify the actual temperature tolerance of *C. capillata* ephyrae and potential interactions with OA.

Materials & Methods

To investigate the effect of CO_2 and temperature on the scyphozoan species *Chrysaora hysoscella* and *Cyanea capillata* we designed a set of experiments with a temperature treatment along a gradient from 4 °C to 31 °C and a CO_2 treatment between 200 and 1000 ppm.

Rearing of polyps and ephyrae

Mature medusae of the scyphozoans *C. capillata* and *Ch. hysoscella* were collected around Helgoland (German Bight, North Sea, 54° 11' N, 7° 53' E) between July and September 2013. Animals were collected in open water, therefore no specific permission was required. All species used during this work were neither endangered nor protected. Medusae were kept in 5 L plastic aquaria filled with filtered (0.2 µm) seawater at 20 °C overnight. After 24 hours, planula larvae which had accumulated at the bottom of the aquarium were collected with a pipette and 5 ml of this planulae solution was added to glass crystallization dishes (8 cm x 4.5 cm, 150 ml) filled with approximately 20 ml filtered seawater. Plastic petri dishes (35mm in diameter) were positioned on the water surface, since the undersides of substrates are preferred for planula settlement (Brewer 1976; Holst and Jarms 2007). After five days in darkness in a 15 °C temperature controlled chamber, the undersides of the plastic petri dishes were densely covered with tiny scyphozoan polyps and the crystallization dishes were filled up to 100ml with filtered seawater and fed with freshly hatched and homogenized nauplii of the brine shrimp, *Artemia franciscana* (Branchiopoda, Sanders Brine Shrimp Company, Morgan, USA). The plastic petri dishes were transferred to clean crystallization dishes filled with filtered seawater after three hours. Polyps were fed every other day until they reached the 8-tentacle stage after about 21 days of feeding. Subsequently, the polyps were fed weekly with living, freshly hatched *A. franciscana. Ch. hysoscella* polyps with an estimated diameter of 0.5 mm were starved for one week before the beginning of the experiment. For each replicate in each treatment eleven randomly chosen polyps from each plastic petri dish were used as one experimental unit (EU);

the other polyps were removed with a blunt curved needle three days before the start of the experiment.

C. capillata polyps used for the ephyrae experiment were cultivated at 15 °C in four 15 L plastic aquaria, filled with 5 L filtered seawater. Each aquarium contained about 40 floating plastic petri dishes colonized with polyps. To initiate strobilation, the temperature was decreased to 5 °C in weekly steps of 2.5 °C. When first strobilations appeared, feeding of the polyps was discontinued, to avoid disturbance of the strobilation process. Ephyrae were collected from the bottom of the aquaria every second day until day 7 after the start of strobilation. At this point about 50 % of the polyps were strobilating. Ephyrae produced in the following 36 hours were used for the experiment. About 200 ephyrae were transferred in a 2 l Erlenmeyer flask with a pipette. The flask was stored at 15 °C in a dark room for 12 hours. The water was slightly aerated to keep the ephyrae in the water column.

Experimental setup

A custom-made aluminium temperature gradient table (Thomas et al. 1963) was used for the experiments. The temperature table was heated on one side and cooled on the other one, which allowed the establishment of a temperature gradient over the table from 2 to 35 °C. The table accommodates 60 glass beakers (800 ml), arranged in 10 columns and 6 rows, allowing a combination of 10 temperature treatments with 6 replicates each. One low and one high CO_2 concentration (200 ppm, 800 ppm in experiment 1 and 200 ppm and 1000 ppm in experiment 2) was used for each temperature treatment, resulting in three replicates for each temperature/CO_2 combination. Seawater-filled plastic cups containing floating EUs (experiment 1, for details see below) or 100 ml Erlenmeyer flasks containing ephyrae in seawater (experiment 2, for details see below) were placed into the glass beakers filled with 200 ml fresh water to provide homogenous temperature conditions in each replicate.

Filtered seawater was used for water exchange after feeding and all EU were bubbled with a CO_2 / air mixture during the experiment. An automatic gas-mixing device (HTC-Hamburg, model: MK 3-2-s) was used to produce the appropriate CO_2 concentration. The CO_2 outflow was controlled twice a day. A pH and total alkalinity (TA) measurement at 15 °C was used to check the CO_2 concentration in the experimental seawater. The pH value was quantified with a ProLab 3000 pH meter with an IoLine pH combination electrode with temperature sensor (type IL-pHT-A170MF-DIN-N). TA was measured from open-cell duplicate potentiometric titration and calculation with modified Gran plots (Bradshaw et al. 1981), with a TitroLine alpha plus titrator equipped with an IoLine pH combination electrode with temperature sensor (type IL-

pHT-A120MF-DIN-N). Certified Reference Material (CRM, Batch No. 104, Scripps Institution of Oceanography, USA) was used for measurement corrections. With the software CO2Cal (Robbins et al. 2010), the carbon dioxide pressure was calculated, using the dissociation constant of carbonic acid of Mehrbach et al. (1973), refit by Dickson and Miller (1987), and dissociation constants for H_2SO_4 from Dickson (1990). All low CO_2 treatments were carried out using air with 200 ppm CO_2. This was done to mimic pre-industrial levels of carbon dioxide, whereas the high CO_2 treatment consisted of bubbling air with either 800 (polyps) or 1000 (ephyrae) ppm CO_2, values close to the predicted end-of-the century ones in several scenarios (IPCC 2014). The CO_2 bubbling was controlled twice a day; water temperature was monitored every other day. A successful test run of the system without test organisms was conducted for seven days before the start of the experiments.

Experiment 1: Polyps

To investigate the effect of temperature and CO_2 on the growth of *Ch. hysoscella* polyps, a temperature gradient from 10 °C to 31 °C was adjusted resulting in ten temperature treatments (10 °C, 12 °C, 15 °C, 16.5 °C, 19 °C, 20 °C, 23 °C, 24.5 °C, 29 °C, 31 °C) and two different CO_2 concentrations (200 and 800 ppm) for each temperature treatment.

Each EU was placed on the water surface of a 100 mL plastic beaker filled with 100 mL filtered seawater (Salinity 33 ± 1) and covered with a lid. A vent in the lid allowed continuous CO_2/air mixture bubbling through glass pipes and limited the gas exchange with the atmosphere. The seawater in the plastic beakers was aerated continuously with 200 or 800 ppm CO_2 (~150 bubbles min^{-1}) during the entire experimental period. Polyps were fed freshly hatched nauplii of *A. franciscana* at 5.5 nauplii mL^{-1} added to the seawater overnight every other day. The next morning, EUs were transferred to beakers filled with pre-temperatured filtered seawater, which had been aerated for 24 h with either 200 or 800 ppm, hermetically sealed and kept for another 24h at either 10 °C or 31 °C. Before using, the water was mixed to obtain the temperature needed in the different treatments (± 0.1 °C). Three polyps of each EU were labelled with a paint marker on the dry upper side of the floating plastic petri dish to allow repeated diameter measurements of the same individuals at the starting date, every sixth experimental day, and on the final day of the experiment. The experiment ran for 24 days and subsequently the polyps were harvested and dried for carbon content measurements.

In an additional experiment the ingestion rate $polyp^{-1}$ day^{-1} was investigated. The experimental set up was similar to experiment 1 with a focus on higher temperatures. A temperature gradient from 20°C to 29.5 °C was adjusted, resulting in eight temperature treatments (20 °C, 21 °C,

22.3 °C, 22.8 °C 24.5 °C, 25.5 °C, 28 °C, 29.5 °C). Polyps were fed overnight every other day with freshly hatched nauplii of *A. franciscana* with a concentration of 0.5 nauplii mL^{-1} obtained from a prepared stock solution. The next morning, the uneaten nauplii of each treatment were counted.

Experiment 2: Ephyrae

The effects of CO_2 and temperature on *C. capillata* ephyrae were tested in a temperature gradient from 4.4 °C to 25.5 °C at ten temperatures (4.4 °C, 5.9 °C, 8.7 °C, 10.2 °C, 13.2 °C, 14.9 °C, 18.2 °C, 20.2 °C, 22.9 °C, 25.5 °C). The CO_2 concentration in this experiment was 200 ppm for the low CO_2 treatment. For the high CO_2 treatment the concentration was raised to 1000 ppm to amplify an OA effect. A total of 72 ephyrae without malformations (as >8 marginal lappets or deformations of lappets) were selected from a 2 L Erlenmeyer flask using a stereomicroscope (Olympus; SZ18) equipped with a camera system. Each selected ephyra was transferred to a smaller Erlenmeyer flask (100 mL) filled with 80 mL filtered seawater which was used as one EU. Continuous bubbling with CO_2/air mixture (50 – 150 bubbles min^{-1}) through glass pipes created a moderate water circulation in the flask allowing continuous swimming activity and food intake of ephyrae. EUs were kept covered with plastic film to reduce gas exchange with the ambient atmosphere. Ephyrae were fed freshly hatched *A. franciscana* nauplii every other day (30 nauplii mL^{-1}) for 3 h. Subsequently, the ephyrae were transferred with a pipette into a clean Erlenmeyer flask filled with preheated/-cooled seawater, as described above. For measurement, each ephyra was transferred into a petridish and photographed in the relaxed state at the start of the experiment and every other day. The ephyra diameters measured between two opposite rhopalia tips (r-diameter) (for details see Hols and Laakmann (2014)) were determined from measurements on the photographs. The experiment ran for eight days. Ephyra were sampled, measured and dried for subsequent carbon measurements. The experiment was conducted twice, identically, resulting in six replicates per treatment.

Analytical procedure

The gelatinous body of polyps or ephyrae has no stabilisation structures, making the measurement of body size inaccurate. To produce reproducible data for polyp sizes, we measured the polyp diameters in the maximum contracted state (for details see Lesniowski et al. (2015)). We correlated the natural logarithm (ln) of a polyp's diameter and the ln of the carbon content. Diameter as size variable was chosen because photographs of contracted,

spherical polyps used for measurements were taken from above resulting in a circular 2 dimensional area with uniform diameter. In comparison to polyps, ephyrae were not poked but photographed when completely relaxed. However, although ephyrae were tested in the same way, because of the disparate length of symmetrically arranged rhopalar lobes, smallest and biggest rhopalar diameter were used as the two measurements needed for area calculation. The ln of an ephyrae's size in area and the ln of the carbon content were used for the regression.
A CHN analyser (elementar, vario MICRO CUBE) was used to obtain the carbon content data. Individuals (n=18) used for analysis were first rinsed in distilled water, and then collected in tin-caps and dried at 60 °C for 24 h before being analysed. Carbon content of polyps and ephyrae was measured at the beginning of the experiment after 1 week of starvation from a random subset of the available animals and at the end of the experiment after 1 day of starvation. Growth rates (gr, d^{-1}) were calculated using the exponential growth equation ($C_t = C_0\, e^{gr\Delta t}$), which was rearranged for gr as follows: $gr = (\ln C_t - \ln C_0) / \Delta t$, where C_t describes the carbon content after the experiment and was calculated with the initial carbon content (C_0) using an exponential equation to the power of the growth rate 'gr' multiplied by the interval between the initial and the final measurement.

Statistical analysis

The relationships between, firstly, carbon content and size for polyps and ephyrae, and, secondly, carbon content and growth rate at the end of the experiment for polyps, were tested by linear regression. Analysis of the diameter change of the polyps and area of ephyrae through the duration of the experiment was assessed with a two-way (temperature and CO_2) repeated measures (RM) ANOVA followed by a Tukey's HSD post hoc test. The effects of treatments on carbon content and growth rate at the end of the experiment were analysed by generalized linear models (GLM), using temperature as a continuous factor, and CO_2 as a non-continuous one. Polyps' *A. franciscana* ingestion was analysed using a two-way ANOVA. All data were tested for normality and homogeneity of variance before statistical tests; no transformations were necessary. All statistical analyses were performed using Statistica 9.1 (StatSoft, Inc.).

Results

For polyps, there was a highly significant ($P < 0.001$) linear regression between the ln of the diameter and the ln of the carbon content, with a positive slope of 1.7 and $r = 0.771$ (Fig 2a). For ephyrae, the linear regression of the ln of the area and the ln of the carbon content was also highly significant ($P < 0.001$), with a positive slope of 0.836 and $r = 0.632$, and shows that our measuring method is also suitable for usage as a response variable for growth of ephyrae (Fig 2b).

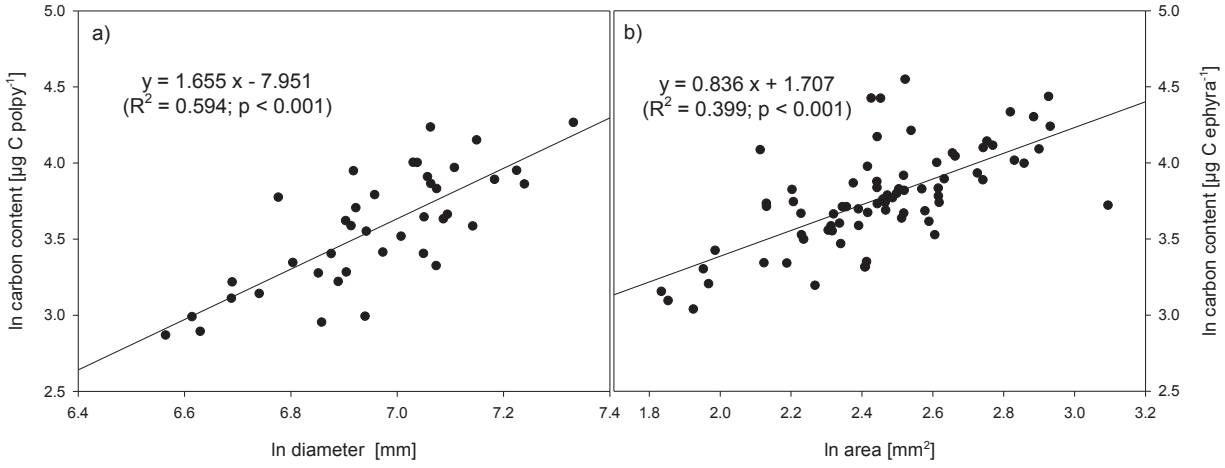

Figure 2: Linear regression of the natural logarithm of the carbon content ((a) µg C polyp^{-1}; (b) µg C ephyra^{-1}) with the natural logarithm of the mean diameter ((a) [mm]) and size ((b) [mm^2]) per replicate.

Experiment 1: polyps of *Ch. hysoscella*

All polyps of the 29 °C and 31 °C temperature treatments died and couldn't be found in the beaker before capturing the first data for both CO_2 treatments and hence could not be included in the statistical analysis. For all other surviving animals, we observed very significant effects of temperature, with the highest growth – up to 12 mm in diameter – between 19 °C and 23 °C under both CO_2 conditions (Fig 3).

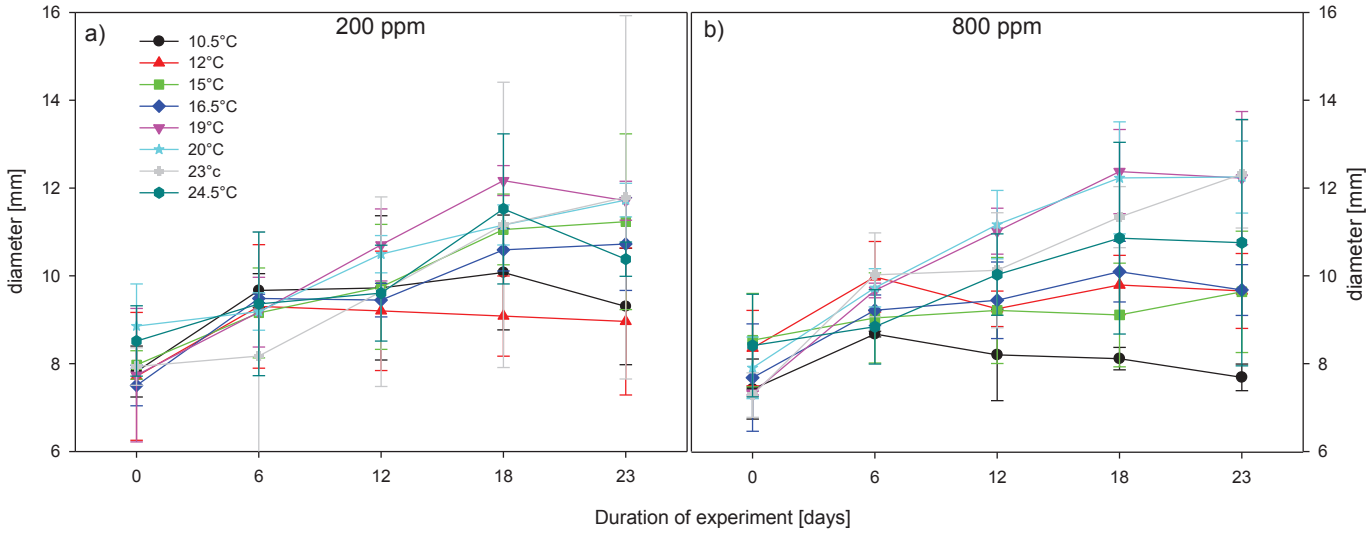

Figure 3: Development of *Ch. hysoscella* polyps as diameter (mean ± SD, mm, n = 3) over time [days] kept in different temperatures for (a) 200 ppm treatment and (b) 800 ppm treatment.

A two-way repeated measurement ANOVA, with diameter as the dependent variable and temperature and CO_2 as the independent variable, showed that temperature and time alone had a significant effect (Table 1).

Table 1: Results of two-way repeated measurement (RM) ANOVA for *Ch. hysoscella* polyps with temperature and CO_2 as independent factors over time and size in diameter (mm) as dependent variable.

Factors & Interactions	SQ	FG	MQ	F	P - value
Temperature	107.61	7	153723	5.384	< 0.001
CO_2	1.81	1	1.81	0.632	0.433
Temp. x CO_2	23.34	7	3.33	1.168	0.352
Time	230.43	4	57.61	120.671	< 0.001
Time x Temp.	86.22	28	3.08	6.450	< 0.001
Time x CO_2	3.58	4	0.89	1.874	0.12
Time x Temp. x CO_2	16.30	28	0.58	1.219	0.232
Error	53.47	112	0.48		

The highest carbon content measured at the end of the experiment (after 23 days) was between 46 - 72 µg C polyp^{-1} (19 - 23 °C) for the 200 ppm treatment and 43 – 53 µg C polyp^{-1} (19 - 24 °C) for 800 ppm (Fig 4a). This is an increase of biomass by a factor 2.8 for 200 ppm and 2.5 for 800 ppm, compared to the starting carbon content of 19 µg C polyp^{-1}. Using a GLM with CO_2 as a factorial variable, and temperature as well as temperature squared to allow for unimodality in the response as predicted from Fig 1., excluded any variables with CO_2, and left only temperature – not even the square temperature ($y = 2.6\ x - 6.21$; $r^2 = 0.80$; $p < 0.001$, Table 2) significant – with the same pattern obviously visible in the analysis of the growth rates. Thus, polyps of *Ch. hysoscella* showed a rapid increase in growth with temperature, and a very steep decline beyond 24 °C, which could not even be captured in growth data in our experiment as the animals all died quickly (Fig 4b).

Table 2: Results of linear regression for polyps of *Chrysaora hysoscella* and of Generalized Linear Model (GLM) for ephyrae of *Cyanea capillata* at the end of the experiment using CO_2 and temperature as independent factors and carbon content (*Ch. hysoscella* polyps [µg C polyp^{-1}]; *C. capillata* ephyrae [µg C ephyra^{-1}]) as dependent variable

	Factors & Interactions	FG	MQ	F	P - value
Polyp (*Ch. hysoscella*)	**Temperature**			57.43	<0.001
Ephyra (*C. capillata*)	**Temperature**	1	235.78	5.86	0.03
	Temperature^2	1	405.66	10.07	0.01
	CO_2	1	7.81	0.19	0.66
	Error	14	40.27		

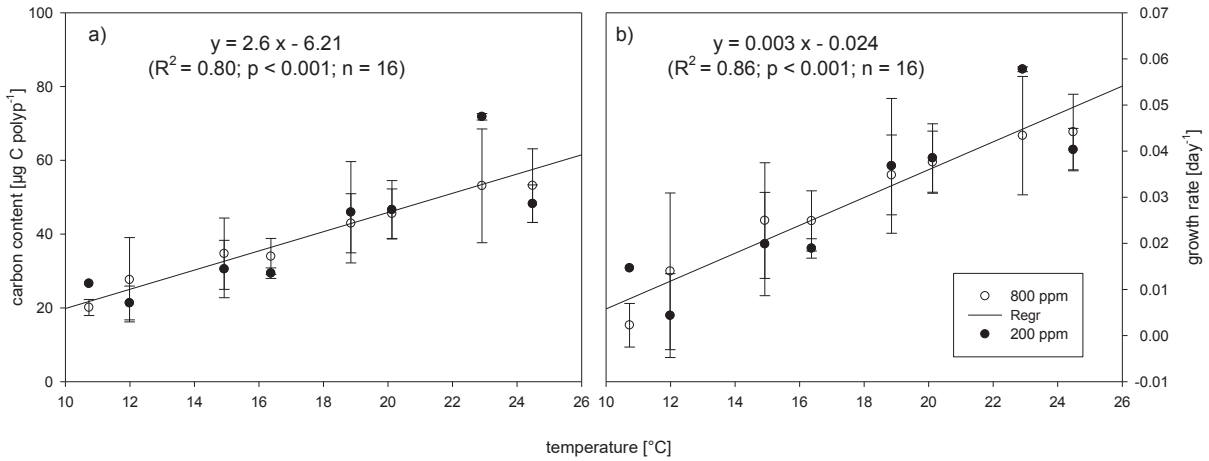

Figure 4: Carbon content and growth rate for polyps of *Ch. hysoscella*
(a) Mean carbon content (± SD, µg C polyp^{-1}) and (b) mean growth rate based on carbon content (±SD, day^{-1}) for low CO_2 treatment (filled circle, 200 ppm) and high CO_2 treatment (circle, 800 ppm) over temperature (°C) for polyps of *Ch. hysoscella* at the end of the experiment (23 days). Regression line was adjusted using the mean values. (n = 18)

Ingestion rates (polyp^{-1} day^{-1}) were constant for both CO_2 treatments from 20 to 25.5 °C (4.5 ± 0.4) for 200 ppm and (4.5 ± 0.5) for 800 (Fig 5). With increasing temperature from 25.5 to 29.5 °C the ingestion rate decreased by a factor of 6.9 to 0.62 for 200 ppm and by factor 5.1 to 0.89 ingested *A. franciscana* polyp^{-1} day^{-1} for 800 ppm at 29.5 °C. There was no significant effect of CO_2 but a highly significant effect of temperature (two-way ANOVA, $P < 0.001$, Table 3).

Table 3: Results of two-way ANOVA for *Ch. hysoscella* polyps with temperature and CO_2 as independent factors over time and ingested *A. franciscana* per polyp as dependent variable after one day.

Factors & Interactions	SQ	FG	MQ	F	P - value
Temperature	95.86	7	13.7	56.677	< 0.001
CO2	0.1	1	0.1	0.415	0.523
Temp. x CO2	1.67	7	0.24	1.003	0.442
Error	10.45	44	0.24		

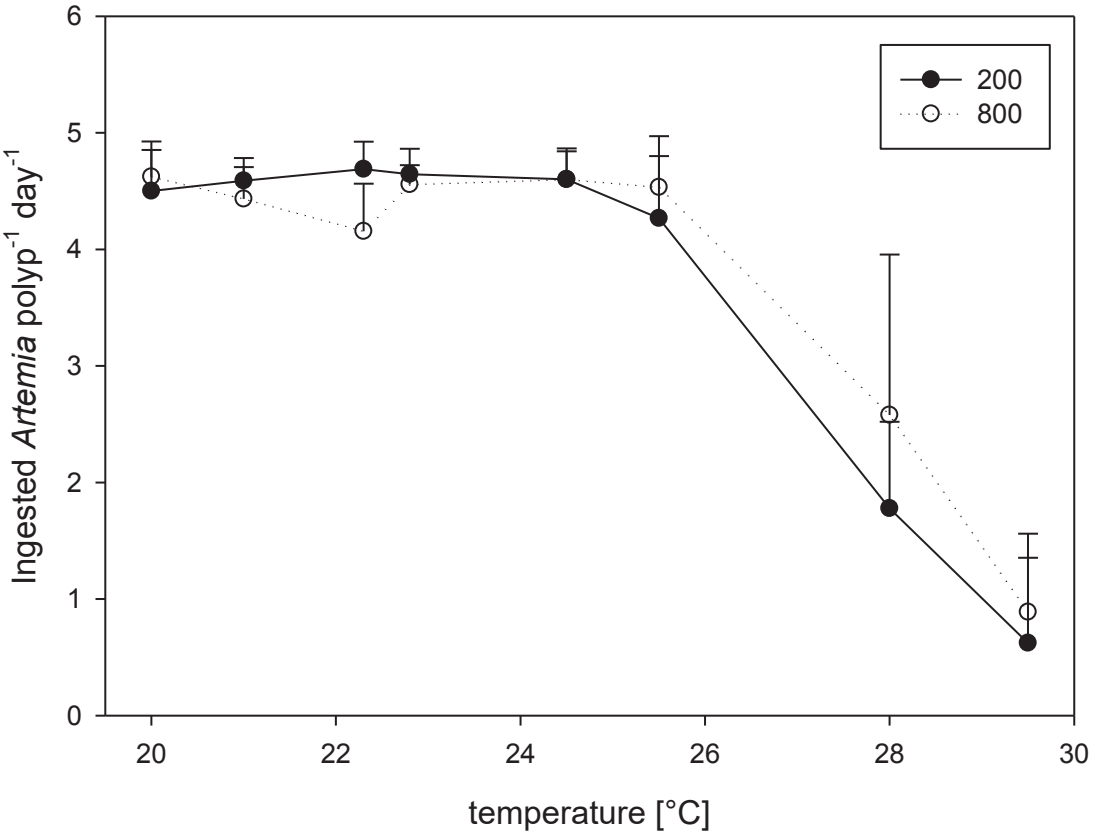

Figure 5: Ingested *A. franciscana* per polyp of *Ch. hysoscella*
Mean ingested *Artemia* polyp^{-1} day^{-1} kept in low (filled circle) or high (circle) CO_2 concentration over temperature [°C]. Positive error bars for low CO_2 and negative error bars for high CO_2 concentration. Error bars: Standard deviation.

Experiment 2

Animal condition of *C. capillata* ephyrae deteriorated by the first measurement when reared in temperatures 22.9 °C and 25.5 °C in both CO_2 treatments. All ephyrae of the 25.5 °C treatment died before the first sampling. For the two highest temperatures, no individuals survived until end of the experiment in the 1000 ppm treatment. The mean (± SD) size in area of ephyrae of *C. capillata* was 1.7 times larger (11.6 (± 3.3) mm^2) at the end of the experiment compared to 6.9 (± 1.6) mm^2 at the beginning for the 200 ppm treatment (Fig 6a). Ephyrae of the 1000 ppm treatment grew during 8 days by a factor of 1.8 from 6.6 (± 1.6) mm^2 to 11.8 (± 2.2) mm^2 (Fig 6b).

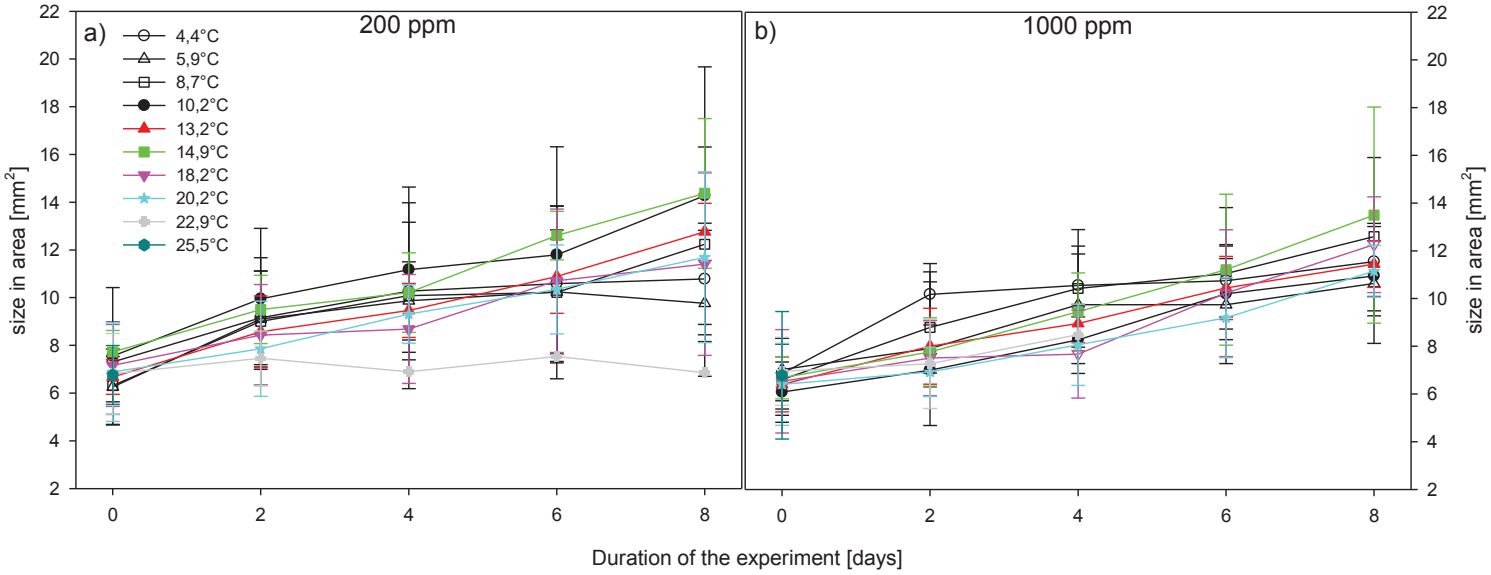

Figure 6: Development of *C. capillata* ephyrae over time
Development of *C. capillata* ephyrae as size in area (mean ± SD, mm^2, n = 6) over time [days] kept in different temperatures. (a) 200 ppm treatment and (b) 1000 ppm treatment.

There was no significant effect of temperature and CO_2 on the increase in area [mm^2] over experiment duration, but a high significance of the interaction between temperature and time (RM-ANOVA, Table 4).

Table 4: Two-way RM ANOVA for *C. capillata* ephyrae with temperature and CO_2 as independent factors over time and size as area (mm^2) as dependent variable.

Factors & Interactions	SQ	FG	MQ	F	P - value
Temperature	53.92	7	7.70	0.362	0.921
CO_2	44.51	1	44.51	2.090	0.153
Temp. x CO_2	74.37	7	10.62	0.499	0.832
Time	1034.29	4	258.57	159.064	< 0.001
Time x Temp.	111.48	28	3.98	2.449	< 0.001
Time x CO_2	6.59	4	1.65	1.013	0.401
Time x Temp. x CO_2	27.50	28	0.98	0.604	0.944
Error	390.14	240	1.63		

After 8 days of the experiment, ephyrae of both CO_2 treatments had a similar mean carbon content of 41.1 (± 16.5) (µg carbon ephyra^{-1}) for 200 ppm and 39.8 µg (± 14.2) (carbon ephyra^{-1}) for 1000 ppm, with temperature having a significant effect (Fig 7a; Table 4; GLM; $p < 0.05$). Ephyrae held in the low CO_2 concentration had the highest mean carbon content at 4.4 °C (53.5 µg C ephyra^{-1}), declining to 20.8 µg C ephyra^{-1} at 22°C. Polynomial regression analysis showed a significant decrease of carbon content with increasing temperature (Fig 7a, polynomial regression (2nd Order), $y = -0.15\ x^2 + 3.16\ x - 30.49$; $r^2 = 0.62$; $p < 0.001$). Between 5 °C and 20 °C, the growth rate after 8 days fluctuated between 0.007 and 0.52 for the low CO_2 treatment and between 0.009 and 0.038 for the high CO_2 (Fig 7b). For both treatments, the growth rate became negative when 20°C was reached with a significant negative relationship between growth rate and temperature (Fig 7b, Polynomial Regression (2nd Order), $y = -0.0005\ x2 + 0.01\ x - 0.02$; $r^2 = 0.72$; $p < 0.001$).

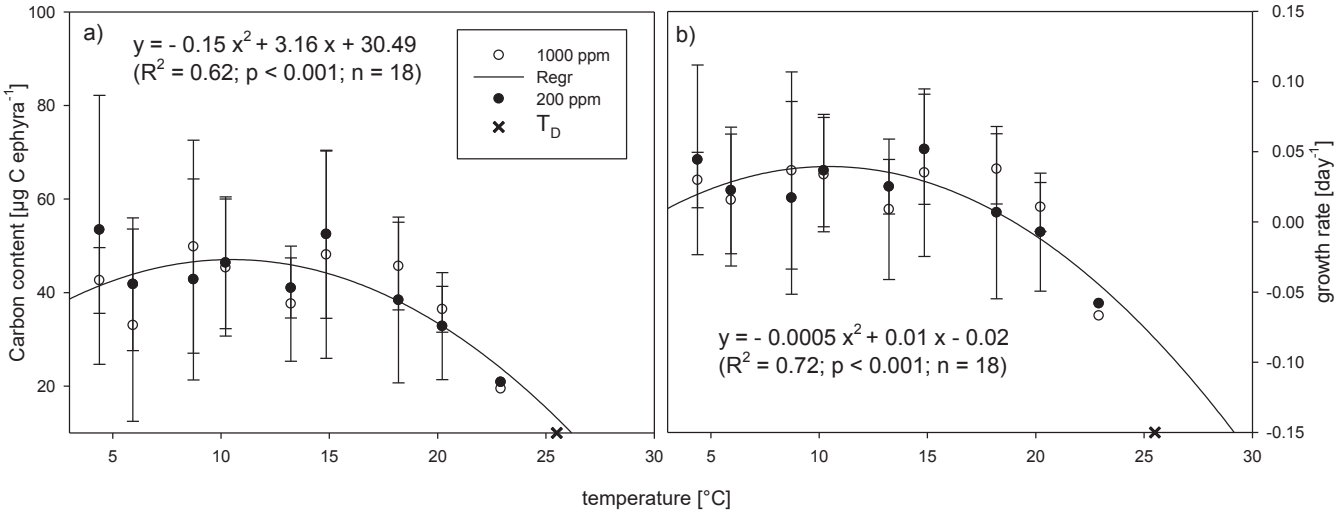

Figure 7: Carbon content and growth rate for ephyrae of *C. capillata*
(a) Mean carbon content 8 days into the experiment (± SD, µg C ephyra^{-1}) and (b) mean growth rate based on carbon content (±SD, day^{-1}) for low CO_2 treatment (filled circle, 200 ppm) and high CO_2 treatment (circle, 1000 ppm) over temperature (°C) for ephyrae of *C. capillata* at the end of the experiment (8 days). Regression line was adjusted using the mean values. Temperatures at which the polyps died at are marked with a black cross. (n = 18)

Discussion

In this study, we observed that temperature has a significant effect on the growth of both juvenile stages of the scyphozoans investigated. In contrast, we observed no effects of CO_2 on polyps of *Ch. hysoscella* or ephyrae of *C. capillata*, nor any interactions of CO_2 with temperature on growth. In summary, no changes in the thermal window could be observed as a result of the additional CO_2 stressor for the life stages investigated.

High CO_2 can affect marine life, most prominently those species that depend on calcium carbonate. However, jellyfish statoliths composed of calcium sulphate hemihydrate, which are used for orientation and balance of scyphozoan and cubozoan medusae (Soetje et al. 2011), can also potentially be affected by high CO_2. Winans and Purcell (2010) reported that statoliths were smaller when ephyrae were cultivated in seawater with a low pH (manipulated with HCl), and statoliths of polyps of the cubozoan species *Alatina* nr *mordens* were 24% smaller at pH 7.6 than those reared at pH 7.8 and 7.9 (Klein et al. 2014). Thus, especially for the ephyra stage, we expected a narrowing of the thermal tolerance window with an increase of CO_2 concentration, as proposed by Pörtner (2008). Potentially as a result of the affected statoliths as well as the increased stress, with the related increases in respiration. However, we found no indications that this may be the case for the species investigated. One reason for this effect not materialising could be that the framework as proposed by Pörtner is incorrect or not applicable to Scyphozoans. On the other hand, many researchers have observed the narrowing of the thermal window under additional stress and it is very unlikely that this fundamental mechanism would work for other species but not for jellyfish. So, if we accept that stress should affect the size of the thermal window, and we observe that this is not the case for the species investigated in our study, we have to conclude that increased CO_2 levels, at least at the realistic near-future levels that we used here, do not affect *Ch. hysoscella* polyps or *C. capillata* ephyrae. In fact, the CO_2 (and resulting pH levels) in the studies cited above were clearly outside this realistic range. Our results showing high tolerances of scyphozoan polyps and ephyrae to high CO_2 are in accordance with previous studies showing that *A. labiata* polyps survive and reproduce at a low pH of 7.2 (Winans and Purcell 2010) and that *Aurelia aurita* ephyrae are only affected by CO_2 at very high levels and only in combination with oxygen stress (Algueró-Muniz et al. 2016). Thus, we conclude that scyphozoan polyps and the juvenile medusa stages of Scyphozoa are rather robust in the face of ocean acidification.

To our knowledge, until now no thermal tolerance data have been published for scyphozoan ephyrae and only few for scyphozoan polyps (Cargo and Schultz 1967; Willcox et al. 2007; Liu

et al. 2009; Purcell et al. 2012). Differences between the used regression models that fit best to the growth of polyps and ephyra, may result in different areas on the thermic tolerance curve of Scyphozoans. Our results suggest a linear growth of *Ch. hysoscella* polyps between 10 °C and 23 °C, implying a range on the thermal tolerance curve between the low critical temperature and the high pejus temperature. At 24.5 °C the polyp growth rate decreased and at 29 °C all polyps died. These findings indicate that temperatures higher than 23° may have negative effects on growth and survival of the of *Ch. hysoscella* polyps, however, predicted temperature and CO_2 increases in the North Sea will probably not affect the polyps. Perennial benthic scyphozoan polyps have to withstand temperature differences between 4°C in winter and 18°C in summer in the North Sea. A high thermal adaption capacity is necessary to survive these differences in full functionality and to grow and reproduce annually. The wide temperature tolerance of polyps corroborates the assumption that the polyps are the more resistant stage in the life cycle of scyphozoans while the medusa stage of most temperate species disappear during colder seasons (Lucas et al. 2012). Higher *Ch. hysoscella* medusa abundances observed after mild winters led to the assumption of higher polyp survival in warmer winters (Merck 1989) and laboratory studies indicated that increasing temperatures may even have positive effects on polyp strobilation, since a higher ephyra production was observed at warmer (10°C) winter temperatures compared to colder (5°C) winter temperatures (Holst 2012a). We therefore suggest more positive than negative effects of current warming trends in the North Sea on the development of *Ch. hysoscella* populations.

Ephyrae of *C. capillata* reared for 8 days in a temperature range from 4.4°C to 25.5 °C in our experiments showed an almost constant growth rate of about 0.027 in the temperature range of 4.4 °C to 13.2 °C, reflecting the optimum temperature range for ephyra growth. In contrast, temperatures warmer than 13.2 °C inhibited the growth and caused death at 25.5 °C. Transferring the results onto the thermic tolerance curve, 13.2 °C is the pejus temperature for *C. capillata* ephyrae. It is not possible to determine the critical temperature (Tcrit) from our results, but since all ephyrae disintegrated at 25.5 °C, Tcrit can be estimated to be within the range of 13.2 °C to 25.5 °C, confirming the assumption that *C. capillata* ephyrae are adapted to colder temperatures and may suffer in warmer temperatures. Our results are in accordance with monitoring in field studies on *C. capillata* ephyrae in the Gullmar Fjord (Gröndahl and Hernroth 1987). In Gullmar Fjord, a first occurrence of ephyrae was recorded in March at temperatures of about 2°C but only slight diameter increases from 1.2 to 1.6 mm were observed by early May at temperatures < 5°C, whereas an exponential ephyra growth to about to 10 mm

in diameter was documented by late June at temperatures from about 5 to 14°C (Gröndahl and Hernroth 1987). Similar to our findings of negative effects of warm temperatures on ephyrae growth, the inhibiting effects of high temperatures were also documented for the strobilation of *C. capillata* polyps in laboratory experiments, revealing that strobilation never occurs at consistently warm temperatures (15°C) but was frequent after a temperature decrease to 10°C or 5°C winter temperature (Holst 2012a). In conclusion, increasing temperatures in the North Sea may have negative effects on the development of *C. capillata* populations.

For an overall statement on the thermic tolerance of scyphozoan juvenile stages based on our results, it must be noted that in our study we were working with two different species and for each species we have investigated just one metagenetic stage: polyps for *Ch. hysoscella* and ephyrae for *C. capillata*.

Our results indicate that different species and life stages show different responses to environmental changes, demonstrating the need for investigations into the various life stages of each species in order to understand the complex role of jellyfish in our marine ecosystems. Furthermore, our results indicate that realistic future CO_2 scenarios might not affect scyphozoan jellyfish.

Chapter III

Effects of water current on growth and survival of polyps of the scyphozoans *Aurelia aurita*, *Chrysaora hysoscella*, *Cyanea capillata* and *Cyanea lamarckii*

Abstract

Man-made changes to the coastlines and the shelf sea are changing the topography with unknown effects for marine life. Increasing numbers of anthropogenic structures are providing new hard substrate and changing bottom water movement, a development that may affect marine benthic organisms. Until now there have been no studies investigating the effect of water current on the benthic stage of the metagenetic life cycle of scyphozoan jellyfish, the polyps stage. To investigate the effect of water current on survival and somatic growth of scyphozoan polyps, both laboratory (Exp 1) and open water experiments (Exp 2 & 3) were conducted. In laboratory experiments, the fitness and survival of scyphozoan polyps were inhibited by high current velocity (> 15 cm s^{-1}). Survival and growth was significantly negatively affected by the grade of exposition and, even under flow-protected conditions, polyp survival was significantly reduced in open water. We conclude that distribution and survival of scyphozoan polyps is affected by water currents and that increasing anthropogenic changes to the coast line and the shelf sea, providing additional settlement substrate and current-calm areas, will be beneficially for scyphozoan jellyfish.

Introduction

Human impact on coastal areas is already acute and is continuously increasing. Apart from the obvious global human interference with marine environments, like pollution and overfishing, human activity has also considerably altered the marine habitat on a regional scale. Eutrophication and other anthropogenic inputs have a great impact in many coastal seas, but humans have also changed the physical structure of the coast. Close to the coast, land reclamation, coastal protection and the deepening and widening of shipping channels have impacted coastal topography, while offshore structures such as wind farms and oil and gas platforms create artificial hard-substrate islands in many sandy shelf seas.

The consequences of these undertakings for coastal ecosystems are still largely unexplored. Many of these human activities increase the amount of hard substrate in coastal areas, providing an extended habitat for those organisms that depend on hard substrate, such as ascidians and many mollusc species. Most scyphozoan jellyfish species depend on a benthic stage for a successful life-cycle. The metagenetic scyphozoan life cycle includes a sexually reproductive medusa stage and an asexually reproductive polyp stage. Pelagic medusae release planula larvae which settle on hard substrates and metamorphose into benthic polyps. Polyps strobilate and produce ephyrae that develop into mature medusae and close the life cycle. Considering between 58,000 and 800,000 planula are released by a single female medusa (Schneider 1988; Lucas and Lawes 1998; Ishii and Takagi 2003; Goldstein and Riisgard 2016), and up to 30 ephyrae are produced per polyp (Purcell et al. 2012), it can be assumed that juvenile benthic stages have a considerable influence on the success of medusa populations. Mass jellyfish occurrence affects many economic sectors, such as tourism, the power supply industry, aquaculture and fisheries (Graham 2001; Ramsak and Stopar 2007; Lo et al. 2008). The factors that influence bloom formation in gelatinous zooplankton are manifold, and most studies mention global warming, eutrophication and overfishing (Mills 2001) as favouring jellyfish, with an ongoing discussion about whether jellyfish blooms are currently increasing globally (Mills 2001; Brotz et al. 2012; Condon et al. 2013). The increasing availability of substrate suitable for settling planula larvae might also affect bloom dynamics (Holst and Jarms 2007). While medusa populations have been shown to be strongly dependent on hydrodynamic currents (Johnson et al. 2001; Barz et al. 2006), the availability of data on the distribution of the rather cryptic polyp stage is very limited (Gröndahl and Hernroth 1987). Hence, there is a great need to increase our knowledge on polyp growth and survival in the context of hydrodynamic properties. For example, water flow, a potential mechanical stressor, has been

shown to affect distribution, growth and reproduction of different alga species (Zwerschke et al. 2013; Molis et al. 2015). Water movements can have positive effects on hydrozoan polyp growth (Boero 1984; Bollens et al. 2001) and on the extension of tentacles in anthozoans polyps (Bell et al. 2006), but nothing is known about the effect of currents on the benthic stages of scyphozoans.

Scyphozoan polyps have no stabilization structures or other exo-/endoskeleton, which makes them very susceptible to being deformed as a result of drag and shear forces produced by water currents. Physical stressors like strong currents affect food capture, reproduction and survival. Benthic scyphozoan polyps are filter feeders dependent on the food particles – mostly zooplankton – passing by in the water column. Tentacles of relaxed polyps are passively expanded until they experience mechanical disturbance or contact with a particle/prey. The effect is an energy consuming tentacle contraction (Passano and McCullough 1964) to transport actively the captured food particle to the polyp's mouth for digestion.

Benthic filter feeders rely on currents for a steady supply of food but if current velocities are too strong this could damage the organisms. The effect of current as a physical stressor on the polyps' survival is important as it influences food availability and therefore the polyps' performance. With appropriate environmental conditions, increasing artificial substratum could be a key factor in the recruitment of scyphozoan polyps in new territories. In laboratory experiments, without any influences of water movement and therefore no physical stress, Holst and Jarms (2007) have already showed that planula larvae prefer artificial to natural substrates for settlement and polyp development. In the field, the settlement site is of great importance for the successful survival of scyphozoan polyps and ephyra production and therefore for jellyfish blooming. Thus, this study set out to investigate the effects of currents on the performance of scyphozoan polyps in combined lab and field experiments.

Materials & Methods

Simulation of different flow regimes under laboratory conditions

To investigate the influence of current velocities on the polyp stage, polyps were exposed to four different current velocities in an annular flow channel. Active effects of current velocity on polyp behaviour were described as tentacle elongation and contraction s^{-1} (tentacle elongation s^{-1}). As increasing value for tentacle elongation s^{-1} implies negative effects of current

on polyps, as more energy is wasted for contraction (Bell et al. 2006). Female larvae-carrying medusae of *Cyanea capillata* (Linnaeus 1758) and *Aurelia aurita* (Linnaeus 1758) were collected during 2010 and 2011 in Kiel Bight and Mecklenburg Bight (Baltic Sea, Germany), respectively. Released planula larvae were cultivated in filtered seawater (15 PSU, 15 °C) with glass slides as settlement substrate and stored for ~10 days in the dark. After metamorphosis into polyps, polyps were fed once weekly with excess amounts of stage II nauplii of the brine shrimp *Artemia salina*. After each feeding, water was exchanged with fresh filtered seawater and polyps were replaced into dark, temperature-controlled rooms.

Polyps (>6 months post settlement) were exposed to different constant flow speeds in an annular flow channel using particle image velocimetry (PIV). PIV is a visual method to describe local fluid velocities by recording the position of small tracer particles introduced into a flowing system over time. The system is composed of an optically transparent test-section, an illuminating light source (laser), a recording medium, and a computer for image post processing (Prasad 2000). Images and video data were analysed with DaVis Imaging Software (LaVision, Version 7.2).

Polyps hanging from glass slides were exposed to current velocities of 0, 5, 10, 20 and 30 cm/s by regulating the voltage applied to a change-pole gear DC motor (RS 320-590, 12 V, 100 rpm; cf. diploma thesis by J. Goldstein, 2012). The tolerance capacity of polyps for each flow regime was assessed video-microscopically by counting the number of polyps detached from substrate plates and the number of contraction-expansion events per polyp and time unit (s^{-1}).

Survival and growth of polyps influenced by currents in open water

Mature medusae of *Cyanea lamarckii* (Péron & Lesueur, 1809) were collected around the island of Helgoland (Germany) in summer 2014. Those medusae that carried planula larvae were kept overnight in 5 L plastic aquaria filled with seawater. After 24 hours all released planulae were collected and transferred to crystallization dishes (150ml) filled with 50 ml of filtered seawater. Polyp colonisation on artificial substrates had already been investigated by Brewer (1976) and Holst and Jarms (2007), therefore PVC-plates (5 x 5 cm) were used as settlement substratum, transferred to the crystallisation dishes (8 cm × 4.5 cm, 150 mL volume) and filled with filtered seawater. Settlement-plates were 3mm thick and with surfaces made rougher. Planulae were kept 7 days at 15 °C in darkness to allow a dense settlement on the plates. After settlement, the plates were transferred to fresh crystallisation dishes and fed mashed, freshly hatched nauplii of the brine shrimp, *Artemia franciscana*. Afterward, polyps were fed twice a week for 3h and then transferred to clean crystallisation dishes with fresh, filtered seawater. After reaching the

8-tentacle stage (~3 weeks) polyps were fed a mixture of mashed and living *Artemia* nauplii. Polyps were cultivated like this for 4 weeks until reaching a mean diameter of 240 µm. Before starting the experiment, the total number of polyps on each plate was determined. Settlement-plates had small drill holes in each corner for mounting to handmade frames using cable ties. 7 settlement plates were installed on each frame, representing 7 replicates. Controlling, cleaning, calculating and mounting was done in filtered seawater to avoid drying up and to guarantee intactness of the polyps until exposure to the selected sites.

The research vessel "Aade" was used to determine locations around the island with the most extreme differences in current velocity. Using Aade's flow meter data, three sites were selected: 1) sheltered site with a current velocity of around 0 cm s^{-1} (54.183827 N, 7.889982 E); 2) semi-exposed site (54.173257 N, 7.898732 E) with a current velocity of maximally 5 cm s^{-1}; and 3) exposed site with a current velocity of up to 40 cm s^{-1} (54.191054 N, 7.880724 E).

Due to weather conditions, the set-up could be deployed at only one site per day. Collecting the samples from the different sites was then adjusted to a 3-day-workflow over the duration of the experiment (25 days) to guarantee equal exposure for all experimental units. Frames were mounted at a depth of 1 m with the help of buoys on the top and weights at the bottom. Initial samples were collected after 2 weeks of exposition and then every 7 days, resulting in 3 samplings per site. On each sampling day, seven settlement plates per site were collected by clipping off the frame and directly transferring them to 2.5 litre buckets filled with filtered seawater. Back in the laboratory, substrate plates were checked for epibiota, and polyps were counted. The diameter of 5 randomly selected polyps of each plate was measured by analysing the images taken by a binocular (Olympus; SZ18) equipped with a camera system and using the Software CellSens Dimension, Version 1.6. Before taking the images, polyps were poked with a blunt curved needle until maximum contraction (Lesniowski et al. 2015).

Effects of protection

Mature medusae of *Ch. hysoscella* (Linnaeus 1767) were collected around Helgoland in September 2011. To cultivate polyps on PVC-plates (4 x 2,5 cm) the same procedure as described above was used. Five plates with polyps were mounted 5 cm apart, one above the other, on a weighted construction that was placed vertically into the water column. Plates used

for the treatment without any protection (open) were placed directly into the water column. Protected treatments were installed inside a plastic bottle (4.5 L) that either had no base to the bottle – allowing water exchange (half-closed treatment) – or a sealed, unmodified bottle filled with filtered seawater (closed treatment). Experimental construction was installed in Helgolands South-harbour (54.177061 N, 7.893159 E) at a 3-meter depth with no current velocity. 224 polyps were determined to be on each plate at the beginning of the exposition time in the lab before bringing them out into open water (n = 8). Polyps on plates were counted every other day during the duration of the experiment (31 days).

Results

Scyphozoan polyps under different flow regimes

Regardless of the current velocity, no polyps were detached from the substrate plates, even at the fastest current speeds of 30 cm s^{-1}. However, polyps showed active movements expressed by rhythmical synchronous contraction and subsequent expansion events with increasing current velocity. *A. aurita* polyps contracted and expanded their tentacles 0.83 times per second in a current velocity range of 0 – 15 cm s^{-1}. As we increased the flow speed to 30 cm s^{-1}, the number of contraction-expansion events increased by a factor of 7.5, reaching 6.22 contraction-expansions per second. Similar patterns of contraction and expansion could be observed in polyps of *C. capillata*, being 6.8 times more frequent in a current velocity range 15 – 30 cm s^{-1} than in velocities 0 – 15 cm s^{-1} (0.35 contraction-expansion events per second). Polynomial regression analysis shows a significant increase in the frequency of contractile behaviour with increasing current velocity (Fig.1. Polynomial regression analysis (3rd Order), *A. aurita* (a): $y = 0.0002x3 + 0.017x^2 + 0.06x + 0.574$; $r^2 = 0.81$; $p < 0.001$; *C. capillata* (b): $y = 0.0002x3 + 0.002x^2 - 0.045x + 0.1$; $r^2 = 0.5$; $p < 0.01$), while there was no significant difference between the two species investigated. The results showed that there was very little change in the behaviour in currents of up to c. 10 cm s-1, with strong increases beyond that.

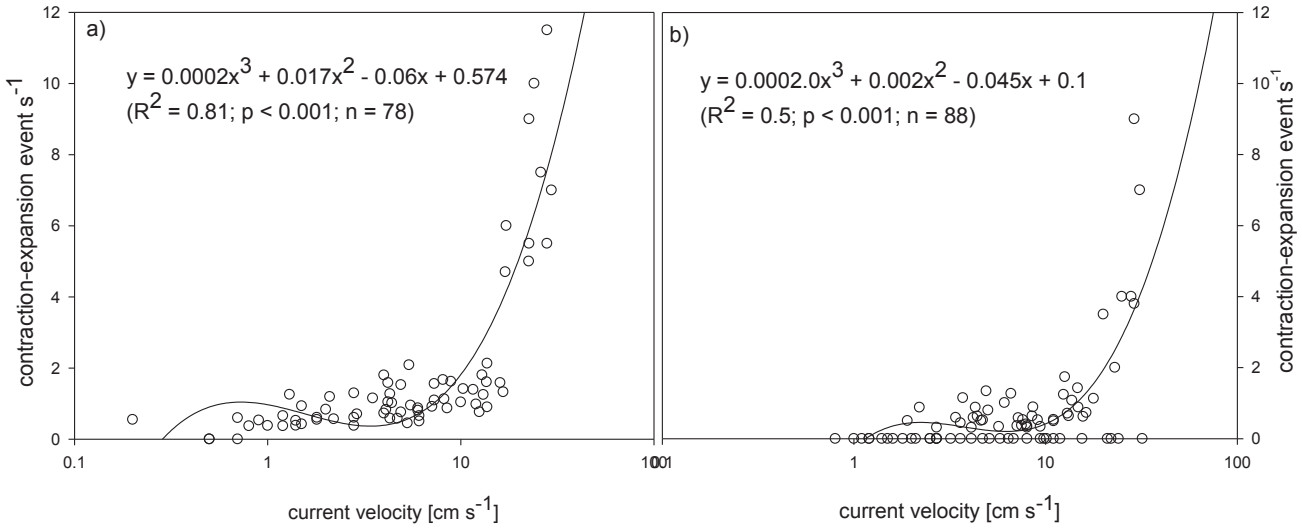

Figure 1: Contraction-expansion events per polyp versus current velocity (cm s^{-1}) for polyps of *Aurelia aurita* (a) and *Cyanea capillata* (b).

Survival and growth of scyphopolyps in open water

For the exposed sampling site, no data is presented, as all polyps disappeared before the first sampling after 11 days of exposition in open water. At the other two sites, 66 % (sheltered) and 86 % (semi-exposed) of the polyps had disappeared before the first sampling (after 11 days of exposition, Fig. 2).

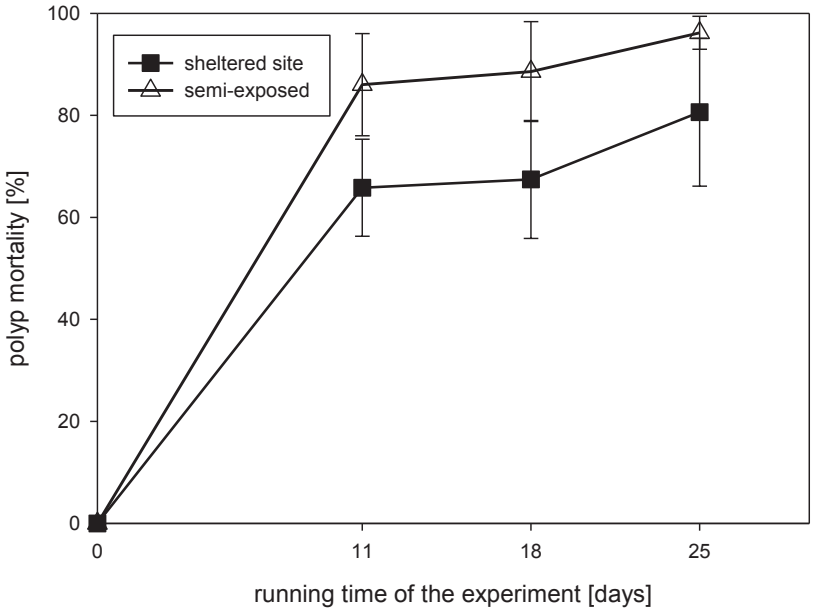

Figure 2: Mean mortality [%] of *Cyanea lamarckii* after 11, 18 and 25 days of exposition in open water at sheltered (black square) and semi-exposed (triangle) site. *Error bars* standard deviation (n = 7).

In total, 42 (± 20.65) of 125 (± 65) polyps at the sheltered site and 18 (± 12) of 134 (± 62) at the semi-exposed site survived for >11 days in open water. After 25 days in open water, the percentage of polyps that had disappeared increased to 81 % at the sheltered site and 96 % at the semi-exposed site. The level of exposure of the site had a significant effect on polyp growth (2way-ANOVA, $P < 0.01$, Table 1, Fig. 2). The mean diameter of semi-exposed polyps of *C.*

lamarckii was 239.1 µm (±15.1) at the beginning and 255.9 µm (± 16.97) at the end of the experiment, corresponding to an increase in diameter of 16.8 µm (7 %) in 25 days.

Table 1 Results of two-way ANOVA for polyps of *Cyanea lamarckii* for experiment 2 (survival and growth in open water) using time and site as independent factors and diameter (µm) as dependent variable.

Factors and interactions	***df***	**MS**	***F***	***P***
Time	2	7759.675	4.759	0.015
Site	1	99999.760	61.332	<0.01
Time x site	2	10243.758	6.283	< 0.01
Error	35	1630.467		

In comparison, the final diameter of polyps at the sheltered site had increased by 177.5 µm (74 %) from 239.1 µm (±15.1) to 416.7 µm (± 11). After 25 days, polyps from the sheltered area were 1.6 times larger in diameter than polyps from the semi-exposed site, indicating a significant effect of the exposure of the site on polyp growth (RM-ANOVA, $P < 0.001$, Table 2, Fig. 3).

Table 2 Results of one-way RM ANOVA for polyps of *Cyanea lamarckii* using site over time as independent factors and diameter (µm) as dependent variable

Factors & Interactions	***df***	**MS**	**F**	**p**
Time	2	15355	3.485	< 0.05
site	1	224566	56.4	< 0.001
Time x site	2	24169	5.485	< 0.01
Error	62	4406		

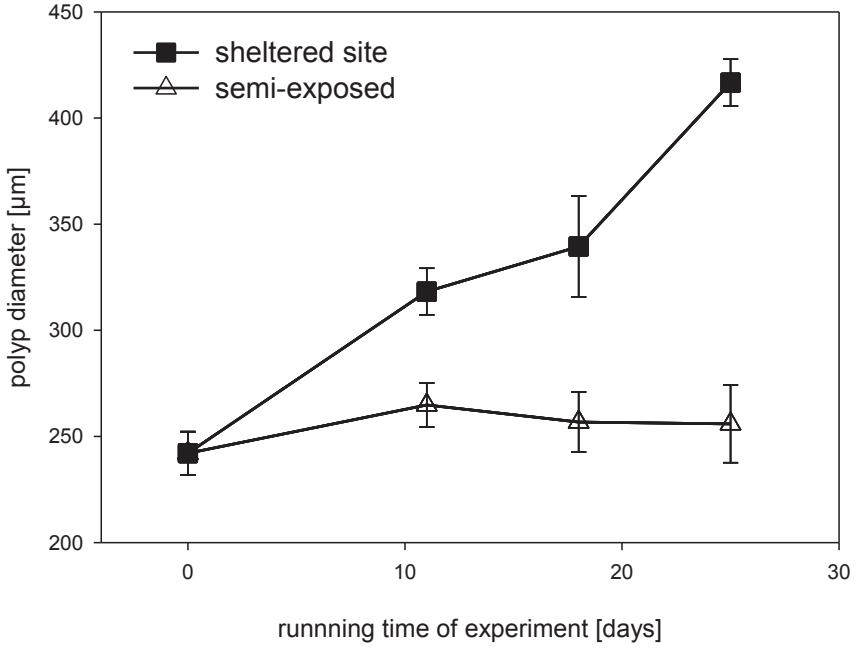

Figure 3: Mean (±SD) diameter of *C. lamarckii* polyps (n = 21) over time exposed to a sheltered site (black squares) or semi-sheltered site (triangles) in open water.

Polyps in flow-protected areas

Polyps reared directly in the water column (open) or in closed containers (closed) showed a similar increase in polyp mortality in the first 17 days of exposure. Initially 31 % for the open treatment and 25.2 % for the closed treatment after 4 days of exposure, polyp mortality increased up to 88 % (open) and 91% (closed) after 17 days. All polyps in the closed container treatment had died by day 19, with a mortality of 99.3 % and 100 % 3 weeks before the end. The mortality rate for the open treatment was 92 % by day 19 and increased steadily until the end of the experiment to 96.9 %. Mean mortality of polyps in the half-closed treatment was 42 % in the first 17 days of exposure, which was 0.7 times lower than the mean of both other treatments during the same duration (58.7 %). By day 19, polyp mortality in the half-closed treatment was 76 % and by day 25 it was <90 %, reaching a maximum of 91.7 % at the end of the experiment. Even under flow-protected conditions, polyp survival was therefore significantly reduced in open water (two-way ANOVA, $P < 0.001$, Table 3, Fig. 4).

Table 3 Results of two-way ANOVA for *Chrysaora hysoscella* polyps for experiment 3 (effects of protection) using time and protection as independent factors and mortality [%] as dependent variable

Factors and interactions	*df*	MS	*F*	*P*
Time	13	6135.5	45.01	< 0.001
Protection	2	2543.1	18.66	< 0.001
Time x protection	26	40.3	0.3	0.999622
Error	99	136.3		

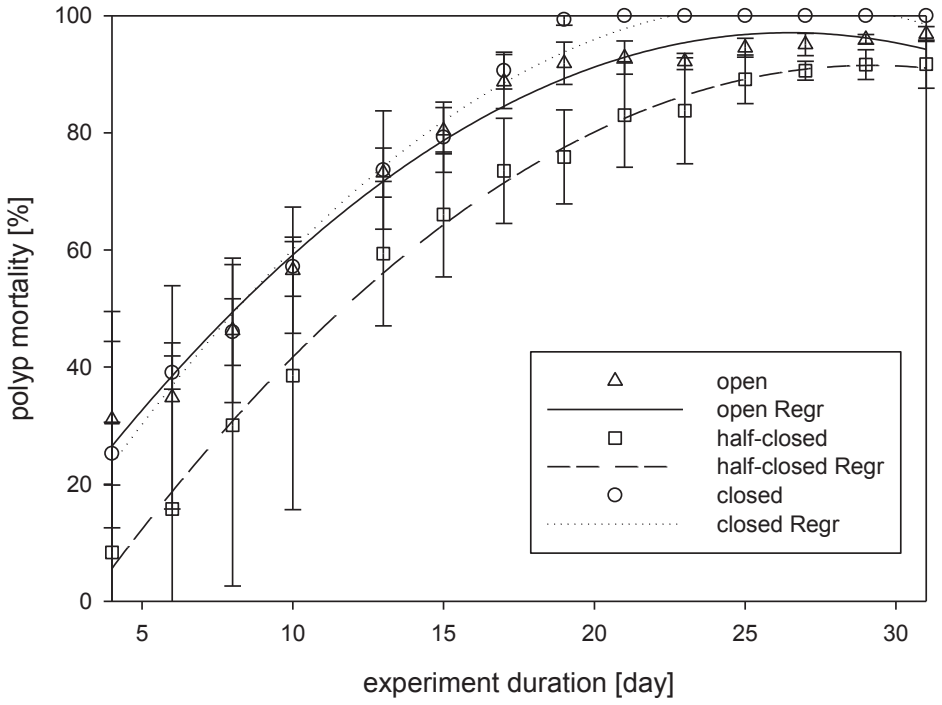

Figure 4: Mean (±SD) mortality [%] of *Chrysaora hysoscella* polyps over time [days] reared in open water (South harbour, Helgoland; current: 0 cm s^{-1}) directly in the water column (triangle; open; n = 6), in half-closed plastic containers (squares; n = 3) and in closed containers (circles; n = 2).

Discussion

Our study indicates that the occurrence and distribution of scyphozoan polyps are strongly dependent on hydrodynamic currents. Benthic filter feeders are dependent on pelagic zooplankton and floating organic particles that are transported in the water column. Interestingly, in our study we observed that despite this need for water flow, growth and survival were greatest when current velocity was lowest. The present results suggest that high current velocity inhibits the fitness and survival of scyphozoan polyps.

Puccinelli et al. (2016) highlighted the effects of biogeography and water currents on 3 different benthic filter feeders. Mechanical and physical stressors influence the distribution of larvae in marine ecosystems. Especially for cnidarian planula larvae, the choice of settlement area is very important. Water currents influence the recruitment, settlement and metamorphosis of scyphozoan planula larvae of *A. aurita* (Keen 1987). After choosing a suitable location on the substrate, the larvae metamorphose into sessile polyps and are completely exposed to environmental influences. The aim of our first experiment was to investigate the effect of different current velocities on scyphozoan polyps. Polyps have an extreme resistance towards shear forces of high flow speeds, since none of the polyps detached from substrate plates. Active movement is expressed by rhythmical synchronous contractions (active, energy consuming) and expansions (passive). Once contracted, the polyp extends its tentacles into the water column where they are exposed to ambient flow conditions. Polyp tentacle contractions are triggered by mechanical and chemical stimuli (Chapman 1965; Loeb and Blanquet 1973). These automatic processes are mechanically coordinated due the simplified diffuse neuronal net and are not controlled by the polyp (Chapman 1965). Our results show an increasing contraction frequency, implying negative effects of flow for the polyp, since contractions require energy (Bell et al. 2006). Our results reveal a significant effect of current velocity on polyps' behaviour in both scyphozoan species. Increasing current velocity means higher mechanical stress, leading to a higher frequency of tentacle contraction by the polyps and, most likely, increased energy consumption. In comparison, polyps exposed to low current velocities primarily contract their tentacles and body when triggered by a mechanical stimulus caused by particle or prey contact. Thus, we conclude that high current velocities have negative effects on scyphozoan polyps, especially above the threshold of ~10 cm s^{-1}, where the frequency of contraction-expansion events increased.

Flow influences the diet of benthic filter feeders, and the extent of this effect depends on the frequency and intensity of the water currents (Puccinelli et al. 2016). In regions with high

current velocities, the limiting factor may be the quality of food and the ability to capture food. Further investigations are needed to find out how polyps' growth and development is influenced by different current velocities with a focus on food capture mechanisms.

All polyps from the exposed treatment died between exposure and the first sampling. All plates were completely overgrown by tubes of amphipods of the species *Jassa marmorata*. Fouling communities on artificial structures at Helgoland are characterised by amphipod species of the genus *Jassa*. Especially at the exposed site used in our investigation, the species *Jassa marmorata* was highly abundant with 500 ind./100 cm^2 compared to the other two species which occur around Helgoland, *J. falcata* and *J.herdmani*, (Beermann 2014). The current velocity at the exposed site was 40 cm s^{-1}, exceeding the flow regimes we investigated in our laboratory experiments. It is therefore difficult to say whether polyps were detached as a result of the strong mechanical impact of current velocity and wave movement, whether they were displaced by the aggressive and much larger amphipods when settling on the PVC-plates, or whether they died because of the very high energy demand related to the very high current velocities. Most likely, the combination of the three made our exposed site an unsuitable habitat. Polyps' mortality at the two other experimental sites increased in the first 11 days of the exposure and was subsequently steady until the end of the experiment. The massive loss of polyps at the beginning could possibly be the adaption to the field environment, leading to detachment of the polyps. Polyps at the sheltered site (0 cm s^{-1}) grew significantly better compared to polyps at the semi-exposed site (5 cm s^{-1}), which had no increase in diameter during 25 days. This result confirms our laboratory experiments, indicating that current velocity has negative consequences on the growth and survival of scyphozoan polyps, even at velocities in a range of 5 to 10 cm/s. At the sheltered site with a current velocity around 0 cm s^{-1}, the polyps doubled their diameter during 25 days. At this site, polyps can potentially invest most of their energy resources into growth, as tentacle contractions are only necessary in the context of food uptake. Based on the assumption that polyps at the investigated sites have the same energy supply, individuals at the semi-exposed site have no resources left for increasing in biomass. Polyps exposed to water movement with a current velocity of 5 cm s^{-1} or higher might run all physiological processes responsible for development and growth at a minimum to save energy and nutrients for survival. Although there are no data available for the sheltered site, at the semi-exposed site the tube-building species *J. marmorata* occurs with an abundance of 250 ind./100 cm^2 (Beermann 2014). PVC-plates from the semi-exposed site had few tubes with living individuals and there were no tubes on plates from the sheltered site. Additionally, the community structure at the different exposure sites could have an effect on the mortality of the

polyps. This experiment should be repeated with an additional experimental site with a current velocity of 10 cm s^{-1} to investigate whether there is a threshold for capturing food.

Successful settlement and metamorphosis of planula larvae does not guarantee survival of a scyphozoan polyp population. The present study indicates negative effects of flow speed on the growth and survival of polyps. Nevertheless, besides the overall flow regime at a certain exposure site, the position in which a polyp is attached to a substratum in the open water is of great importance. Besides decreasing flow speed near the bottom and the resulting forces, the level of protection at a settlement site influences positively or negatively the performance and survival of sessile benthic polyps. This was demonstrated in our study by decreased mortality for polyps under protected conditions.

For the half-closed treatment, the bottom of the bottle was replaced so polyps had access to fresh water and therefore to food throughout the experiment's duration. Our assumption is that the bottle wall protected polyps from predation and settlement as there was no direct way for other organisms to reach the polyps. To make contact with the polyps, predators had to find the opening of the bottle. Polyps of the "open"-treatment were floating in the water column, attainable for various endemic adult and juvenile organisms. This treatment was exposed to predators and accessible for settlement by others. With an abundance of 200 ind./100 cm^2, the different species use the experimental units (PVC-plates) as additional substratum for a new habitat and replaces weak competitive species. Hoover (2012) highlighted that predation of scyphozoan polyps by nudibranchs is an underappreciated population-controlling factor. This must be noted, as for our experiment the experimental units were in the water column, out of reach of other benthic polyp predators. The closed treatment was filled with filtered seawater and polyps were completely protected from water movement, predation and fouling. During the exposure, the water in the closed bottle was not changed and polyps were not fed. Although polyps survive much longer periods without food (own observation), starvation could be the reason for 100 % mortality after 17 days. The significant effect of shelter on polyps' survival makes the whole subtidal area around Helgoland an optimal location for polyp colonies. The topography of Helgoland with its characteristic bedrock and furrows, panels and caves, offers optimal protection for scyphozoan polyps.

Further investigation must be carried out to identify how polyp populations are influenced by current velocities and community compositions at different exposure sites. Repeating the open water experiment at different times of year should be considered to gain a clearer picture of the effects of different species' composition at the experimental site during any given season.

Additionally, other materials for substratum should be investigated for polyp settlement under field conditions.

In conclusion, our results demonstrate that the distibution and survival of scyphozoan polyps are affected by water currents and we expect that increasing anthropogenic changes to the coastal region – and, as a consequence, the creation of new, protected, current-calm settlement substrate – will be beneficial for scyphozoan jellyfish.

General discussion

Jellyfish are a major component of the planktonic food webs of different marine environments, however the role of gelatinous zooplankton – and especially the impact of scyphomedusae on other zooplankton and fish populations in global marine food web systems – is not really understood. Nevertheless, their role should not be underestimated. Besides the negative effects of gelatinous zooplankton blooms for humans (as a result of their impact on tourism, the power supply industry, aquaculture and fisheries), mass occurrence of jellyfish, especially scyphomedusae, can also have a negative effect on marine food webs. A bloom is an increase in individuals and thus biomass of animals as a consequence of seasonal life cycles and/or temporary or transient chemical or physical phenomena (Duarte et al. 2014). During jellyfish blooms, the massive abundance of gelatinous zooplankton may wield a top-down control on zooplankton resources, influencing the food supply of higher trophic levels. Hansson et al. (2005) described the reduction by half of several species of zooplankton in the Danish Limfjord as an effect of predation by *A. aurita*. Also Brodeur et al. (2008) estimated that nearly one third of the zooplankton standing stock in the Bering Sea was consumed by jellyfish during a bloom. Additionally, the presence of jellyfish affects not only planktivorous fish stocks through direct competition for food (Lynam et al. 2005b), but also through predation on juvenile fish stages (Purcell et al. 1994). Möller (1984) and Purcell & Grover (1990) reported that direct predation by gelatinous zooplankton halves cohorts of newly-hatched larval herring (*Clupea harengus*). Simultaneously, huge numbers of jellyfish convert massive amounts of carbon: the carbon included in primary producers is ingested by secondary consumers and, through consumption, converted into gelatinous biomass. As there are only a few species which consume gelatinous zooplankton – e.g. moon fish (*Mola mola*), butterfish (*Peprilus triacanthus*), marine turtles, seals, sharks and some seabird species – the gelatinous carbon is extracted from the natural cycling of carbon and is no longer available for higher trophic levels (Condon et al. 2011). On the other hand, the gelatinous biomass concentrated during blooms binds carbon that is transported to the benthos during the dying off of the bloom, where it is available for microbial communities. The carbon bound in the "jelly-flux" can be decomposed on the way down by planktonic microbial communities or can enrich the nutrient concentration at the benthos for different decomposers.

Nevertheless, the factors controlling jellyfish occurrence must be investigated to understand the role of gelatinous zooplankton in the marine food web. Discussion of jellyfish blooms usually refers to the medusa stage of scyphozoa. Very extreme events from the East Asian Marginal

Seas produce medusae with a bell diameter up to 2 m and a wet weight of ca. 200 Kilogram with negative effects for the fish industry due to more than 2000 medusae per set net per day (Uye 2014). These jellyfish blooms can exceed 10 t wet weight per 100 m^{-3} (Lilley et al. 2011). This thesis investigated the growth constraints of the benthic polyp stage and the pelagic ephyra stage of the metagenetic scyphozoan life cycle. This thesis assumes that the polyp stage is the bottleneck of bloom formation. A successful recruitment by planula larvae, followed by an undisturbed growth and asexual reproduction leading to ephyra-building strobilation, is the basis for the jellyfish bloom of adult medusae. The aim of the investigation of this thesis was to gain a better understanding as to how nutrient concentration, temperature, ocean acidification and water current influence the somatic growth of polyps.

A few studies have investigated the recruitment of planula larvae, showing that planula are dependent on hard substrate (Werner 1984), and in laboratory experiments larvae prefer a synthetic substratum, when offered both natural and artificial substratum (Brewer 1976; Keen 1987; Holst and Jarms 2007). In nature, polyps are found after successful recruitment of the larvae at the surface of piers and floating docks (Kozloff 1983). Laboratory experiments with planula larvae of *A. aurita* show the significant effect of temperature and food on settlement and development (Webster and Lucas 2012). Only if the environmental and ecological factors are suitable do polyps strobilate almost simultaneously, producing ephyrae that develop into adult medusae.

Changes in nutrient concentration as a consequence of eutrophication can influence productivity and the composition of primary communities (Elser et al. 2007). Regarding the effects of food quality and quantity, the results of this thesis show that there is a significant effect of "bad" food on somatic growth of *C. capillata* polyps, but no effect on *Ch. hysoscella* polyps. In my experiments, I compared two limited (N- and P-) food sources offered to the polyps. Theoretically, the highest growth rate for *Ch. Hysoscella* should be reached when the offered food is not nutrient limited. In our experiments, N-limited copepods provided as food had a C:N ratio that is similar to the typical C:N ratio of gelatinous zooplankton (Kogovsek et al. 2014). Additionally, the difference in N-content between nitrogen limited and non-limited copepods was small (Malzahn et al. 2007), too small to detect an effect of N-limited food. Further investigation should be done with a greater difference between N-limited and non-limited food.

The negative growth of *C. capillata* polyps when fed with P-limited copepods follows the growth rate hypothesis. This hypothesis links the phosphorus demand of organisms with their growth rate. High amounts of phosphorus-rich nucleic acids are needed for growth, meaning

growth parameters are negatively influenced when phosphorus availability is limited. Species differences in phosphorus demand could be another reason for the contrasting effects of nutrient limitation for the investigated *C. capillata* and *Ch. hysoscella* polyps, with a negative effect of P-limitation on *C. capillata* polyps but not on *Ch. hysoscella* polyps. Additionally, we observed that the effect of food quality on *C. capillata* polyps is significant, regardless of food quantity. At high food quantity as well as low food quantity, the polyp growth rate is positively affected by the nutrient concentration of the food, whereby growth rate for high quantity treatments is higher than for low quantity treatments. Sterner and Robinson (1994) state that at low nutrient availability organisms need only substances for constructing energy for the metabolic system and that anabolic processes are not of importance. Our results underline a converse theory, confirming studies of Boersma and Kreutzer (2002) and Schoo et al. (2012), who argue that even if the nutrient availability is scarce, resulting in very low growth rates, growth and regeneration processes are continued, meaning a continuous demand for molecular structure components like nitrogen and phosphorus. Regarding our nutrition experiment results, it is easier to understand the success of gelatinous zooplankton, especially in environments where the nutrient concentration is harmful for non-gelatinous organisms.

Concerning the results of OA experiments on juvenile stages of gelatinous organisms investigated here, we can state that CO_2, at least in realistic projection of end-of-century concentrations, has no significant direct effects on polyps of *C. capillata* and *Ch. hysoscella* and ephyrae of *C. capillata*. At the time of writing this thesis, only three laboratory studies had been published investigating the effects of pH on gelatinous organisms. Klein et al. (2014) demonstrates that cubozoan polyps of *Alatina* nr *mordens* tolerate scenarios with low pH, but with a statolith size decreasing by 24 % at pH 7.6 when compared to pH 7.8 and 7.9. Winans and Purcell (2010) show that statoliths of ephyra are smaller when reared in seawater with low pH but that survival and reproduction were not influenced. The newest study, published by Algueró-Muñiz et al. (2016), is in line with the above mentioned studies and states that *A. aurita* ephyrae are robust and will likely not suffer from ongoing OA. These results are predictions for direct effects of rising atmospheric CO_2 and the resulting changes in the seawater chemistry. Absorbed atmospheric CO_2 will decrease the pH value in global oceans and affect marine organisms indirectly. The intake of stoichiometric unbalanced food will challenge many homoeostatic marine consumers. Predicted changes in seawater chemistry and nutrient concentration will influence, for example, the primary producer composition and re-organize the food web and the chemical structure of the food will change. However, based on the results

of this thesis, I conclude that scyphozoan polyps and juvenile medusa stages of scyphozoa will be not affected by ocean acidification.

Furthermore, we investigated the effect of temperature on juvenile stages of scyphozoans and observed a significant effect on somatic growth of polyps of *Ch. hysoscella* and ephyrae of *C. capillata*. In the laboratory, we observed that *Ch. hysoscella* polyps have a linear growth between 10 °C and 23 °C. Sessile benthic polyps must cope with temperature differences from 4 °C in winter to 18 °C in summer in the North Sea, a temperature range of 14 °C. A high thermal adaption capacity is thus necessary for successful survival, growth and reproduction over a wide temperature spectrum. Our results show an adaption of *Ch. hysoscella* polyps to a wide temperature range. Additionally, laboratory experiments indicate that increasing temperature may have a positive effect on polyp strobilation (Holst 2012a). Our results, along with the observation of the disappearance of scyphomedusae during colder seasons (Lucas et al. 2012), lead to the assumption that for scyphozoans, the polyp stage is more resistant to temperature than the medusa stage and especially polyps of *Ch. hysoscella* will benefit from the current warming trends in the North Sea.

Ephyrae of *C. capillata* show the optimum temperature range for growth between 4.4 °C and 13.2 °C, with an inhibited growth at temperatures higher than 13.2 °C and death by 25.5 °C at the highest. Our results underline the study published by Grondahl and Hernroth (1987) from the Gullmar Fjord, presenting a minimal growth in winter for water temperature between 2 and 5 °C and an increase in growth by a factor of 5 when water temperature was between 5 and 14 °C later in the year. Compared to *Ch. hysoscella* polyps, ephyrae of *C. capillata* have a thermal tolerance adapted to colder temperatures, indicating that they will suffer as a result of the predicted temperature increase in the North Sea.

Besides the effects of global warming, it is the anthropogenic impact on coastal regions that could influence the occurrence and distribution of scyphozoan polyps. The increase of anthropogenic artificial substrates due to reshaping of natural coastlines, as well as the increasing number of man-made structures at the coast and in the shelf sea, alters the coastal topography with an unclear effect on benthic organisms. Hence, we investigated the effects of currents on the performance of polyps of the scyphozoans *A. aurita*, *Ch. hysoscella*, *C. capillata* and *C. lamarckii* in combined laboratory and field experiments. The laboratory experiment shows that higher current velocity means higher mechanical stress, leading to an increased frequency of energy-consuming tentacle/body contraction. Polyps of *C. capillata* and *A. aurita* contracted their tentacles approximately 7 times more often when the current velocity was doubled from 15 cm s^{-1} to 30 cm s^{-1}. This significant effect of current velocity on polyps'

behaviour leads to the conclusion that high current velocities have a negative effect on scyphozoan polyps. Polyps on plates were taken to three sites with different current velocities to verify the laboratory results in the field. The results of the field experiment support the laboratory results. Polyps' growth was significantly higher at the sheltered site with a current velocity of 0 cm s^{-1} compared to the semi-exposed site (current velocity: 5 cm s^{-1}), where there was no growth of the polyps. Polyps exposed to current velocities of 5 cm s^{-1} or higher might minimize their physiological processes for growth, development and reproduction to save energy and nutrients for survival. All polyps disappeared at the exposed site with a current velocity of 40 cm s^{-1}. The highest current velocity used in the laboratory experiments was 30 cm s^{-1}, with no polyp detachment from the cultivation plates. One explanation for the observed results could be a velocity threshold for polyp detachment between 30 and 40 cm s^{-1} or the community structure in the field. Experimental plates were completely overgrown by tubes of the amphipod species of the genus *Jassa*. It is therefore difficult to say if the polyps were displaced by the amphipods or detached by the strong mechanical impact of high current velocity. This led to a follow-up experiment, investigating the effect of shelter. Polyps on plates were exposed in open water in a construction simulating three different levels of protection. Based on the results, the assumption is that the position in which a polyp is attached to a substratum in the open water is of great importance. Not only does the effect of current velocity influence the success of scyphozoan polyps, but also the position of a polyp on a substratum, especially the level of protection against predators and competitors. The results underline the significant positive effect of shelter and demonstrate that the availability of food and protection from competitors for settlement and benthic and pelagic predators are the basis of the successful settlement and metamorphosis of planula larvae and polyps' growth, development and reproduction.

Future implications

In light of predicted future scenarios, we have learned that the direct effects of levels of OA realistically projected for the end of the century on gelatinous zooplankton are not likely to be large. Phosphorus concentrations in the North Sea declined in recent decades (Wiltshire et al. 2008), leading to a higher C:P ratio in the phytoplankton and affecting quality and quantity of primary producers and higher trophic levels resulting in the availability of poor food quality. Regarding the results of this thesis, it can be assumed that jellyfish will not be affected by the aforementioned predicted nutritional changes. Global warming and current warming trends for the North Sea will have more positive than negative effects on the development of scyphozoan

population development. Increasing anthropogenic changes to coastlines globally and the growing development of artificial substratum due to wind parks and other human-made structures in the shelf seas are also factors which favour the occurrence and distribution of gelatinous zooplankton.

The success of scyphozoans in the North Sea, especially of the polyp stage, is founded on the high tolerance range to changing environmental conditions.

Outlook

Future research will need to focus on both direct and indirect factors like nutrient depletion, temperature, CO_2 / pH, hydrodynamic stress, intra- and interspecific competition and predation that influence jellyfish occurrence to understand how climate change and other pressures affect jellyfish bloom dynamics. Laboratory experiments investigating temperature tolerance range and the effect level of nutrient depletion for different species and different life stages will help us to determine the bottleneck of population and bloom development. Data from field experiments are completely lacking but will be important in corroborating results from laboratory experiments. Recruitment of planula larvae in open water and the settlement behaviour and development from planula to polyps has never been observed in field experiments and should be investigated. Experiments with individual polyps and colonies with different degrees of shelter and substratum in the field could help us to understand where polyps are settled. The results of this thesis demonstrate the adaption of the juvenile scyphozoan stages to extreme environmental conditions. For a closer understanding of the role of gelatinous zooplankton in the food web and the marine ecosystems, further studies on different life stages of key species will be indispensable.

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Eidestattliche Erklärung

(Gem. § 6(5) Nr. 1-3 PromO)

Ich, Thomas Lesniowski, Düppelstr. 54, 44789 Bochum,
(Vorname, Name, Anschrift, Matr.-Nr.)

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