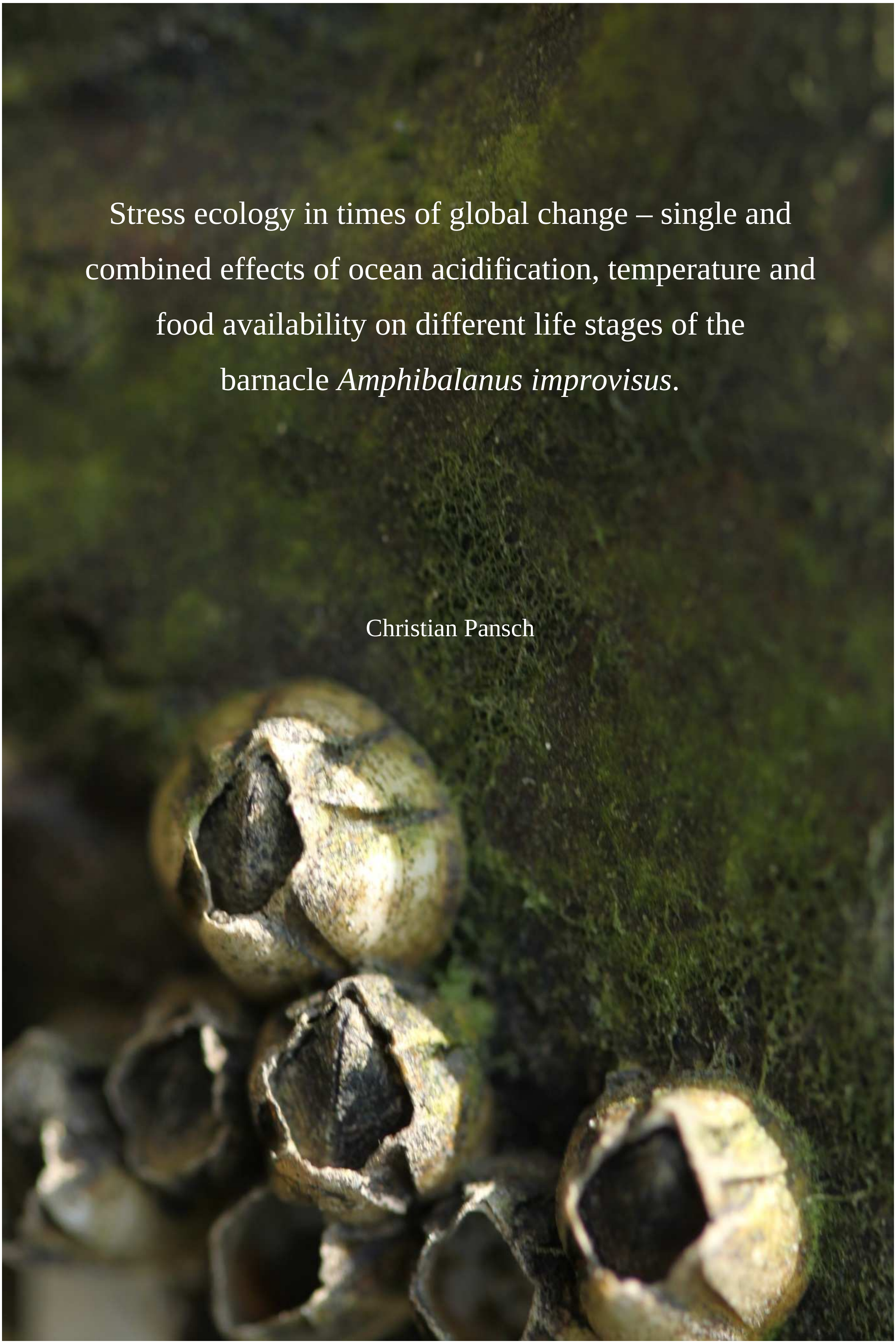

Stress ecology in times of global change – single and combined effects of ocean acidification, temperature and food availability on different life stages of the barnacle *Amphibalanus improvisus*.

Christian Pansch

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Stressökologie in Zeiten des Klimawandels – Einzel- und interaktive Effekte von Ozeanversauerung, Temperatur und Nahrungsverfügbarkeit auf verschiedene Lebensstadien der Seepocke *Amphibalanus improvisus*.

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CONTENT

SUMMARY

Increasing atmospheric CO_2 affects seawater pH and chemistry. This process, commonly known as ocean acidification (OA), has led to a decrease in oceanic seawater pH by 0.1 since the industrial revolution. Oceanic models show that mean pH may fall from the current 8.1 units to 7.8 and 7.5 by 2100 and 2300, corresponding to levels of about 1000 and 2000 µatm pCO_2, respectively. Coastal habitats have been described to differ substantially from open ocean conditions. Not only absolute mean values and annual or daily fluctuations but even future predictions differs considerably from open ocean norms. These characteristics evolved over many years and likely formed species or populations, which are more robust to future OA than species or populations from more stable oceanic environments. Calcifying species as well as early life-history stages of marine organisms are considered to be mainly affected by OA. The acorn barnacle *Amphibalanus improvisus* is a dominant marine calcifier within the western Baltic Sea and has a complex life cycle with various stages such as feeding nauplii and non-feeding cyprids as well as settled calcifying juveniles. Thus, this species is an ideal organism to address a wide range of hypotheses. In this thesis, I investigated the sensitivity of the *A. improvisus* towards OA stress in combination with additional environmental parameters such as temperature and food availability. The organisms for this study came from the Kiel Fjord, Germany and the Tjärnö Archipelago, Sweden, which, to a certain extent, allows interpretations on the population level. As one of the first cases, this study evaluates the entire life cycle of an invertebrate towards OA in combination with additional stressors.

Nauplius larvae of *A. improvisus* were affected neither by moderate (<1500 µatm pCO_2) nor by severe (>1500 µatm) OA under summer temperature conditions of 20 °C in the Kiel population or under slightly elevated temperatures (25 °C) in the Tjärnö population. However, in cooler waters (12 °C) severe OA drastically slowed down the larval development of Kiel individuals. Warming generally increased the survival as well as the rate of development in barnacle nauplii but cypris larvae also suffered increased mortality with increasing temperatures. Cyprid size (Tjärnö) and settlement (Kiel) were unaffected by temperature and OA but survival was enhanced under severe OA in the Kiel population. While survival, growth, condition index, reproduction, shell strength as well as development of the F1 generation of juvenile barnacles from Kiel were rather unaffected by OA over 20 weeks, moulting frequency increased with increasing acidification of the seawater. Net-calcification was reduced under increasing acidification with negative

impacts on the shell maintenance of adults. This was true for barnacles cohorts collected in summer but when a cohort of juvenile barnacles was collected in autumn and investigated under OA scenarios over the winter, severe OA negatively impacted juvenile growth after 10 weeks. Seawater warming by 4 °C temporary effected the growth of juvenile barnacles. It also decreased the condition index and increased the breaking resistance, i.e. led to an increased investment in shell production compared to body growth. Juvenile barnacles from Tjärnö showed a generally higher sensitivity to OA with reduced growth and survival under moderate and severe OA when combined with food limitation and under severe OA when food availability was increased. Food availability in general, seems to be the major factor driving the performance of juvenile barnacles (increasing growth, condition index, reproduction and shell strength), in both the Kiel and the Tjärnö populations.

In the present study, barnacles have been shown to withstand predicted near-future OA well, with if at all, only sub-lethal effects on the different life-history stages. Larval stages of both the Kiel and the Tjärnö populations were tolerant to even the highest OA levels applied (3250 µatm pCO_2, pH 7.5). Nevertheless, the sensitivity of juveniles to OA differs remarkably between barnacle populations. Juveniles of *A. improvisus* from Tjärnö responded more sensitively to OA than juveniles from Kiel. Juveniles of *A. improvisus* from Tjärnö responded more sensitively to OA than juveniles from Kiel. These population-specific differences might be explained by the high natural variability in pH/pCO_2 over the year in the Kiel Fjord and the possibility of barnacles to adapt to these fluctuations over many generations. Assuming that this adaptation is potentially also valid for other barnacle populations, *A. improvisus* from Tjärnö will likely adapt to anthropogenic OA in the future. In conclusion, barnacles might be pre-selected to withstand strong small-scale as well as large-scale fluctuations in their natural environments. Nevertheless, habitat characteristics and thus population specific differences seem to play a role in determining the resilience of *A. improvisus* to OA. Even seasonality likely controls the resilience of this species to OA. Since OA is expected to increase more drastically in shallow coastal habitats in the future, the absolute OA tolerance limits of this species are still to be evaluated in more detail. Additionally, the synergistic effects of OA and factors such as warming or desalination need to be considered when future predictions are made. Community structures depend on the responses of various organisms and there will be "losers" and "winners" in the fate of future OA. Thus, although single species such as *A. improvisus* will be not lethally impacted in a future acidified ocean, community structures are likely to change due to the higher sensitivity of other organisms.

ZUSAMMENFASSUNG

Steigende CO_2 Emissionen verändern den pH im Meerwasser sowie auch dessen Karbonatchemie. Dieser Prozess wird im Allgemeinen als Ozeanversauerung (OV) bezeichnet und hat seit der industriellen Revolution zu einer Reduktion des Meerwasser-pHs um 0,1 Einheiten geführt. Zukunftsmodelle für die Ozeane prognostizieren ein weiteres Absinken des pHs von heutigen 8,1 auf Werte um 7,8 im Jahre 2100 und um 7,5 im Jahre 2300. Diese entsprechen einem Anstieg des Meerwasser- $p\mathrm{CO_2}$s auf circa 1000 und 2000 µatm. Küstenhabitate unterscheiden sich jedoch drastisch von ozeanischen Bedingungen. Nicht nur absolute Mittelwerte oder Fluktuationen im Jahres- und auch im Tagesgang, sondern auch Prognosen für eine zukünftig zu erwartende Ozeanversauerung weichen hierbei stark von „Ozean"-Modellen ab. Diese Eigenheiten von Küstenhabitaten, welche sich erst über die Jahre herausgebildeten, könnten dazu geführt haben, dass sich lokale Arten oder Populationen mit der Zeit durch Selektion an diese Gegebenheiten angepasst haben. Kalzifizierende Arten sowie frühontogenetische Lebensstadien werden in der Literatur hierbei als sehr empfindlich im Umgang mit einer zukünftigen OV prognostiziert. Die Brackwasser-Seepocke *Amphibalanus improvisus* ist ein dominanter Kalzifizierer in der westlichen Ostsee und weist einen komplexen Lebenszyklus mit fressenden Nauplius- sowie nicht-fressenden Cyprislarven und einem siedelndem und kalzifizierendem juvenilen Stadium auf. Somit bietet diese Art die Möglichkeit zur Bearbeitung einer Reihe relevanter Fragen zur OV-Forschung. In meiner Arbeit habe ich die Sensibilität der Seepocke *A. improvisus* auf OV sowie auf OV in Kombination mit zusätzlichen Umweltfaktoren wie Temperaturveränderungen und Futterlimitierung untersucht. Die untersuchten Organismen stammten aus der Kieler Förde sowie aus Gewässern vor Tjärnö in Schweden, wodurch auch Aussagen auf Populationsebene möglich sind. Als eine der ersten Studien, habe ich hierbei die Sensibilität des gesamten Lebenszyklus eines Invertebraten auf OV in Kombination mit zusätzlichen Stressoren untersucht.

Naupliuslarven der Art *A. improvisus* wurden weder von moderater (<1500 µatm $p\mathrm{CO_2}$) noch von starker (>1500 µatm $p\mathrm{CO_2}$) OV beeinträchtigt, wenn sie unter normalen Sommertemperaturen von 20°C (Kiel Population) oder unter leicht erhöhten Temperaturen von 25°C (Tjärnö Population) gehältert wurden. In 12°C kaltem Wasser war die Larvalentwicklung der Nauplien unter OV jedoch stark verlangsamt. Erwärmung förderte das Überleben und beschleunigte die Larvalentwicklung der Nauplien. Cyprislarven

hingegen zeigten eine erhöhte Mortalität unter Erwärmung. Die Größe (Tjärnö) sowie der Siedlungserfolg (Kiel) der Cyprislarven waren von der Temperatur sowie der OV unabhängig wobei OV das Überleben der Cyprislarven begünstigte. Das Überleben, Wachstum, der Konditionsindex, Reproduktion und auch die Schalenstabilität von juvenilen Seepocken sowie die Entwicklung derer F1 Generation blieben von der OV über 20 Wochen hinweg unbeeinflusst wobei die Häutungsfrequenz der Tiere unter OV Bedingungen gesteigert war. Die Nettokalzifizierung war mit steigender OV herabgesetzt was sich negativ auf den äußeren Zustand der Schalen adulter Tiere auswirkte. Diese Beobachtungen waren jedoch nur für Sommerkohorten von *A. improvisus* gegeben. Herbstkohorten von *A. improvisus* die über den Winter im Experiment groß gezogen wurden wiesen ab der 10. Woche unter starker OV reduzierte Wachstumsraten auf. Erwärmung um 4°C hatte kurzzeitig positive Effekte auf das Wachstum der Seepocken, reduzierte den Konditionsindex und erhöhte die Schalenstabilität und führte somit zu einer gesteigerten Schalenbildung im Vergleich zum Weichkörperwachstum. Juvenile Seepocken aus Tjärnö zeigten im Allgemeinen eine erhöhte Sensibilität gegenüber OV. Das Überleben sowie das Wachstum der Tiere waren bei Futterlimitierung unter moderater sowie starker OV und unter normalen Futterbedingungen nur unter starker OV herabgesetzt. In beiden Populationen war die Futterverfügbargeit jedoch bei Weitem die dominierende Komponente in der Entwicklung der Seepocken.

In der vorliegenden Studie wurden Seepocken der Art *A. improvisus* als sehr resistent gegenüber einer zu erwartenden OV gezeigt. Es konnten in den meisten Fällen, wenn überhaupt, nur subletale Effekte unter moderater OV gezeigt werden. Larven aus Kiel und auch Tjärnö zeigten sich sogar gegenüber extremen $p\mathrm{CO_2}$ Behandlungen von 3250 µatm (pH 7,5) resistent, solange die OV nicht in Kombination mit herabgesetzter Temperatur auftrat. Dennoch traten enorme Unterschiede zwischen einzelnen Populationen von *A. improvisus* auf wobei juvenile Individuen aus Tjärnö viel sensibler auf OV reagierten als Individuen aus Kiel. Diese Populationsspezifischen Unterschiede lassen sich möglicherweise auf Anpassungsprozesse der hiesigen Seepockenpopulation an die starken natürlichen pH/$p\mathrm{CO_2}$ Fluktuationen in der Kieler Förde über viele Generationen zurückführen. Unter der Annahme, dass weitere Seepockenpopulationen, wie zum Beispiel die untersuchte Population aus Tjärnö, über ein vergleichbares Potential der Anpassung verfügt wie es für die Kieler Population anzunehmen ist, werden auch weitere Seepockenpopulationen von zukünftiger OV nicht stark beeinflusst sein.

Zusammenfassend scheint es, dass Seepocken generell an starke Schwankungen in Ihrem natürlichen Habitat angepasst sind; seien diese kleinskalig oder großskaliger Natur. Dennoch scheinen Habitat-spezifische Komponenten eine wichtige Rolle in der Resistenz von *A. improvisus* gegenüber OV zu spielen. Es scheint hierbei außerdem auch die saisonale Komponente einen Einfluss auf die Resistenz dieser Art gegenüber OV zu haben. Da die OV für Küstenhabitate in Zukunft jedoch noch gravierender ausfallen wird wie für ozeanische Gewässer, ist es von zentraler Bedeutung die tatsächliche resistenzschwelle von *A. improvisus* gegenüber OV zu untersuchen. Weitere synergistische Effekte wie Erwärmung und Aussüßung der Meere müssen in Zukunftsprognosen über diese Art ebenfalls mit einbezogen werden. Marine Gemeinschaften sind stark von den Resistenzen einzelner Arten abhängig und es wurde über die letzten Jahre gezeigt, dass es Verlierer und Gewinner geben wird. Auch wenn sich die einzelne Art *A. improvisus* als relativ tolerant gegenüber der OV zeigte, sind Veränderungen in den marinen Lebensgemeinschaften unter zukünftiger OV dennoch zu erwarten.

ABBREVIATIONS

A_T	total alkalinity
$CaCO_3$	calcium carbonate
CO_2	carbon dioxide
$CO_{2(aq)}$	aqueous carbon dioxide
CO_3^{2-}	carbonate
C_T	total dissolved inorganic carbon
d.f.	degrees of freedom
DW	dry weight
FW	fresh weight
HCO_3^-	bicarbonate
M	mean
MS	mean squares (variance)
N	number of sample units (replicates)
OA	ocean acidification
Ω	$CaCO_3$ saturation state
pCO_2	carbon dioxide partial pressure
pH_{NBS}	pH at the NBS (National Bureau of Standards) scale
pH_T	pH at the total scale
SD	standard deviation
SE	standard error
SS	total sum of squares

1. General Introduction

1.1. Ocean Acidification

Fossil fuel combustion over the past 200 years has led to an increase in atmospheric CO_2 concentrations. This is widely documented in ice cores as well as in manual measurements initiated by Charles David Keeling in 1956 (Fig. 1.1). While CO_2 concentrations in the atmosphere have remained in the range of 172 to 300 ppm over the past 800 000 years (Lüthi et al. 2008), CO_2 concentrations increased from about 285 ppm to about 390 ppm since the beginning of the industrial revolution. Not only the rate at which concentrations of atmospheric CO_2 increase today but also its amplitude will by far exceed the levels that have occurred throughout the past 2 million years (Lüthi et al. 2008; Gattuso and Hansson 2011).

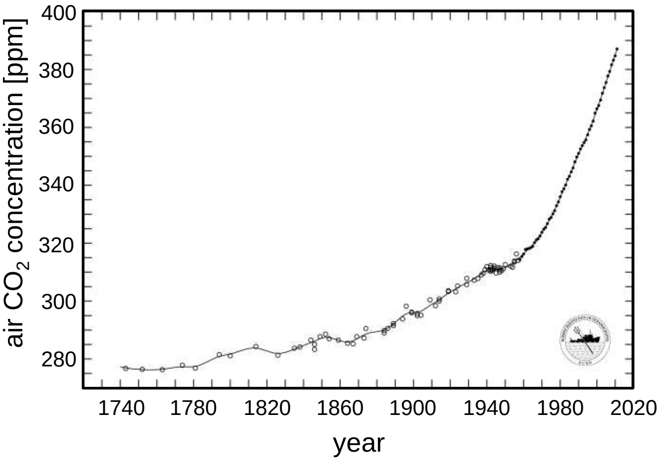

Figure 1.1 Time trend of the concentration of CO_2 in air extracted from an Antarctic ice cores (open black circles) combined with the trend based on direct atmospheric measurements (air average) from Mauna Loa and the South Pole (closed black circles). Last updated January 2012 (from http://scrippsco2.ucsd.edu).

In a wide consortium, the "Intergovernmental Panel on Climate Change" describes a number of future scenarios, largely depending on socio-economic parameters considered as well as on possible mitigation strategies (IPCC 2007). Atmospheric CO_2 concentrations are expected to increase to 650 ppm (B1-Scenario) or even up to 970 ppm (A1-Scenario) by the year 2100 with an actual rate of about 3.4% per year (IPCC 2007; Le Quéré et al. 2009). CO_2 is widely recognized as a greenhouse gas causing global warming. Besides, large amounts of atmospheric CO_2 dissolve in the ocean leading to a reduction in pH of the ocean, a phenomenon commonly referred to as "ocean acidification" (OA) or as the "other CO_2-problem" (Doney et al. 2009). The world's oceans cover about two thirds of the earth' surface and play a crucial role in determining climatic processes. Serving as a carbon sink about one half of the anthropogenically released CO_2 has already been taken up by the oceans (Sabine et al. 2004).

CO_2 dissolves in seawater ($CO_{2(aq)}$) by physical dissolution followed by a complex reaction with other carbonate system species present in seawater. The reaction of $CO_{2(aq)}$

with water molecules forms H_2CO_3 (carbonic acid), which dissociates into HCO_3^- (bicarbonate) releasing a H^+ (proton). This H^+ reacts with CO_3^{2-} (carbonate ions) present in seawater and another HCO_3^- is formed. All carbonate species are in equilibrium with each other as shown in Fig. 1.2. The sum of $CO_{2(aq)}$, HCO_3^- and CO_3^{2-} is called the dissolved inorganic carbon (C_T).

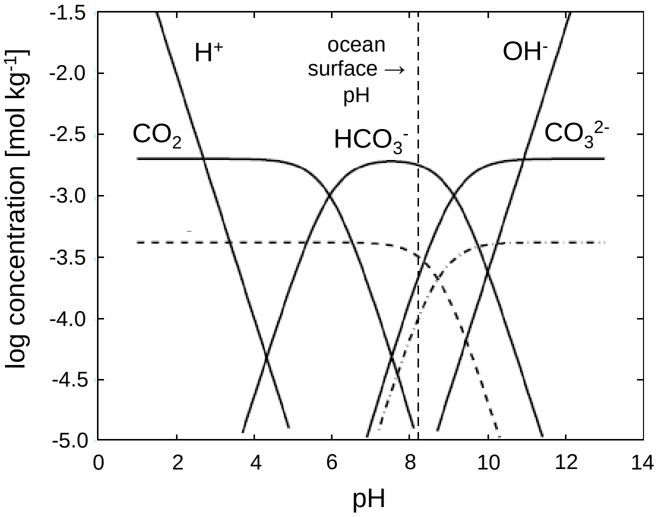

Figure 1.2 Bjerrum plot showing the pH dependency of the three carbonate system species CO_2, HCO_3^- and CO_3^{2-}. The dashed line corresponds to present day mean ocean surface pH (from Zeebe and Wolf-Gladrow 2001).

In the today's ocean only about 0.5% of the inorganic carbon is present in the form of H_2CO_3 and 13% in the form of CO_3^{2-}, while the majority is present in the form of HCO_3^- (>85%; Zeebe and Wolf-Gladrow 2001). With a future increase in atmospheric CO_2 concentrations, overall C_T will increase in the oceans with the carbonate species being shifted towards a relative increase of HCO_3^- (Zeebe and Wolf-Gladrow 2001). Due to a net generation of H^+ by the dissolution of CO_2 in seawater, the oceanic pH decreases (Fig. 1.3). The pH in seawater is a measure of the acidity (pH = –log 10 [H^+]), which is reported on different scales: National Bureau of Standards (pH_{NBS}), seawater (pH_{SWS}), free (pH_F) and total (pH_T). The pH values on the NBS scale are about 0.15 units higher while values on the SWS scale are about 0.01 units lower than pH values on the total scale. The A_T (total alkalinity) is the sum of weak bases balancing the charge differences between anions and cations (= $[HCO_3^-] + 2[CO_3^{2-}] + [B(OH)_4^-] + [OH^-] + [HPO_3^{2-}] + 2[PO_4^{3-}] + [SiO(OH)_3^-] + [NH_3] + [HS^-] \ldots - [H^+]_F - [HSO_4^-] - [HF] - [H_3PO_4] \ldots$), whereas the major component of this buffering system is the carbonate alkalinity with its components HCO_3^- and CO_3^{2-} (= $[HCO_3^-] + 2[CO_3^{2-}]$).

Another consequence of the dissolution of CO_2 in seawater is that the concentration of CO_3^{2-} decreases, lowering the saturation state of carbonate minerals present in shells and skeletons of marine organisms. Three major biogenic $CaCO_3$ (calcium carbonate) minerals are present in marine environments with aragonite being 1.5 times more soluble than calcite. High Mg-calcite (>12% Magnesium) is even more soluble than aragonite (Dickson 2010; Gattuso and Hansson 2011; Ries et al. 2011). Seawater is in equilibrium with the respective mineral when $\Omega = 1$, it is supersaturated when $\Omega > 1$ (promoting inorganic precipitation) and it is undersaturated when $\Omega < 1$ (promoting inorganic dissolution).

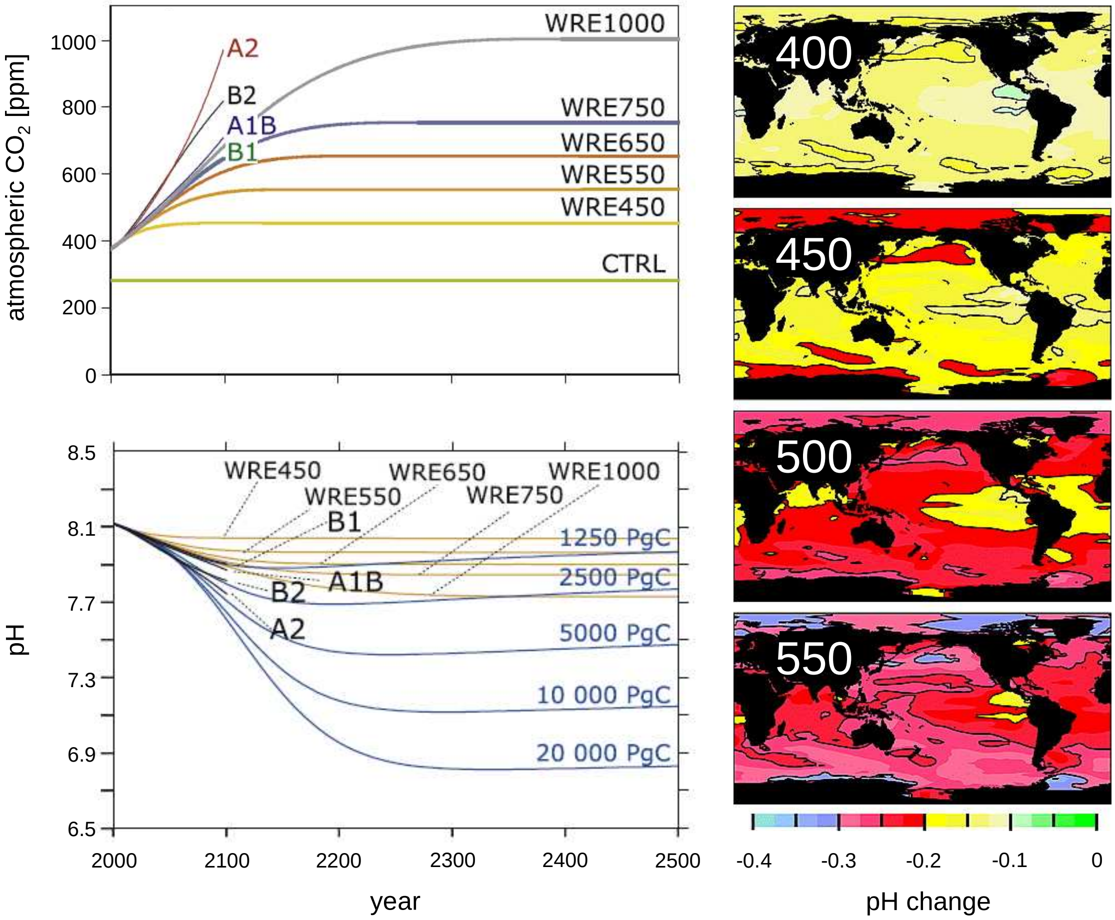

Figure 1.3 Atmospheric CO_2 predicted for the SRES emission pathways and specified for the WRE stabilization Scenarios and predicted Surface ocean pH from ocean model simulations (from Caldeira and Wicket 2005; Cao and Caldeira 2008).

Since the beginning of the industrial revolution, oceanic pH has dropped by 0.1 units to a pH of 8.1. An on-going decrease of about 0.002 pH units per year can be measured at present (Gattuso and Hansson 2011). Future scenarios predict a further decrease in oceanic pH of up to 0.2 units (WRE stabilization scenarios), of up to 0.5 pH units (20 000 Pg C release) by the year 2100 and of up to 0.4 units (WRE stabilization scenarios) and of up to 1.3 pH units (20 000 Pg C release) by 2300 (Caldeira and Wickett 2005; Fig. 1.3). Even more important for marine calcifiers will be the decrease in $CaCO_3$ saturation state, which already has decreased by about 20% since 1766 and is projected to decrease by about 40% by 2100 (Gattuso and Lavigne 2009; Gattuso and Hansson 2011). The magnitude of a decline in $CaCO_3$ saturation states however, varies with latitude and high-latitude oceans will be more affected than low-latitude oceans. Future predictions on the calcite saturation of the surface oceans are presented in Fig. 1.4.

Several processes can influence the carbonate chemistry of seawater (Zeebe and Wolf-Gladrow 2001) as represented in Fig. 1.5. Calcification (i.e. $CaCO_3$ formation) leads to a reduction of A_T (total alkalinity; with a minor decrease of C_T and pH), while $CaCO_3$ dissolution leads to the opposite effects. Both photosynthesis and release of CO_2 from the system ocean lead to a reduction of C_T and an increase of pH (with a minor increase of A_T under photosynthesis), while respiration and CO_2 absorption by the system lead to opposite effects. Thus, in contrast to the open ocean, coastal habitats and estuaries with high biological activity differ substantially with respect to carbonate chemistry as well as annual and even daily natural fluctuations.

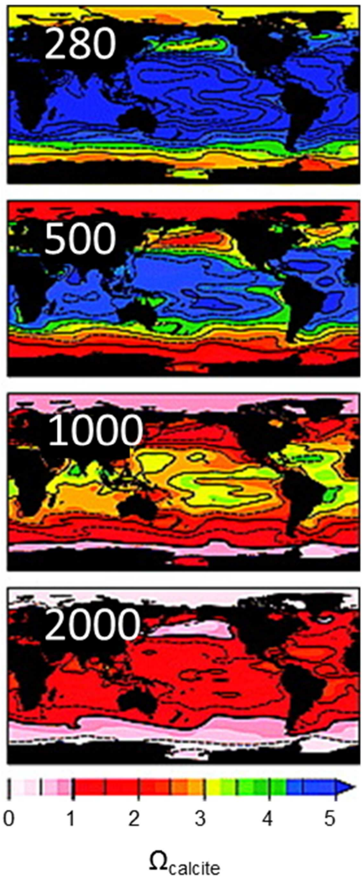

Figure 1.4 Changes in surface ocean pH relative to pre-industrial values and saturation state of calcite for different atmospheric CO_2 stabilization levels (ppm). By 500 ppm, aragonite will dissolve in parts of the near-surface Southern Ocean; by 1000 ppm, calcite will dissolve in most of the high latitude oceans (from Cao and Caldeira 2008).

As presented in Fig. 1.6, pH fluctuations measured over the year in several coastal habitats can be substantial compared to open ocean time series. Not only long term trends have been reported to differ from open ocean predictions (Hinga 2002; Wootton et al. 2008) but also seasonal signals of more than 1 pH unit were revealed (Provoost et al. 2010). Habitats such as a seaweed bed of

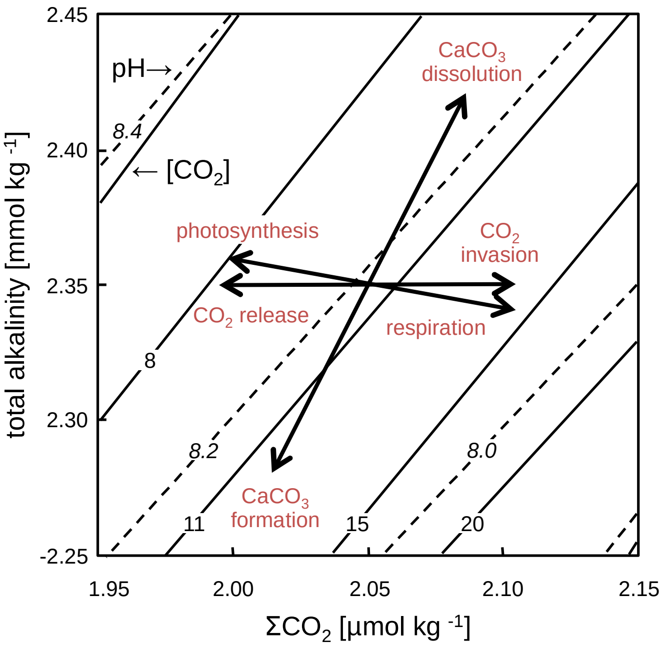

Figure 1.5 Processes affecting seawater carbonate chemistry (values shown refer to T=15°C, S=35, and P=1 atm). Solid and dashed lines indicate contours of constant dissolved CO_2 and pH, respectively (from Zeebe and Wolf-Gladrow 2001).

Fucus spp. dominated by biological activity shows even daily fluctuations of more than 1 pH unit (Vincent Saderne pers. commun.). In highly productive near-shore coastal ecosystems, the effect of eutrophication on carbon cycling can even counter the effect of OA on the carbonate chemistry of surface waters (Borges and Gypens 2010). River discharges of acidic water can further exacerbate acidification in estuaries (Salisbury et al. 2008) while brackish habitats exhibit generally lower salinity values with consequently a lower alkalinity and thus, naturally lower $CaCO_3$ saturation states (Ω) compared to open ocean waters (Hjalmarsson et al. 2008; Miller et al. 2009).

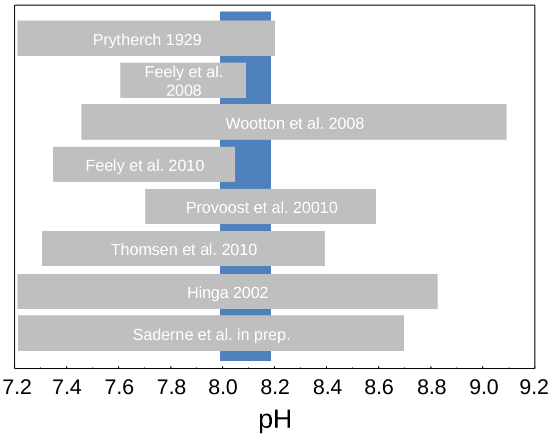

Figure 1.6 Fluctuation in pH reported from diverse coastal habitats worldwide. Vertical blue shaded area represents open ocean fluctuations taken from the Bermuda Atlantic Time Series (Bates and Peters 2007) and Hawaii Ocean Time Series (Dore et al. 2009)

1.2. Biological Impacts of Ocean Acidification

Potential changes in carbonate chemistry of the oceans and possible implications for marine organisms was already discussed in the mid of the 20th century (Rubey 1951). From less than 50 articles discussing the wider subject of “OA” in the late 1990s, the effort on OA research has increased substantially to more than 700 articles treating the subject to date with most effort so far dedicated to phytoplankton, corals and molluscs (Gattuso and Hansson 2011).

OA is widely known to generally affect two fundamental processes in marine animals: the acid-base status and calcification (Gattuso and Hansson 2011). The extracellular (body fluids) as well as the intracellular pH of organisms play an important role in biological processes such as enzyme activity or protein functions. . Highly active organisms such as fish, crustaceans or cephalopods are believed to cope better with OA due to their high metabolic rates and naturally strong fluctuations of pCO_2 in their body fluids (Melzner et al. 2009). A major mechanism to counter extracellular acidosis induced by OA is by active accumulation of HCO_3^- in order to buffer the surplus H^+ in body fluids (Gutowska et al. 2010b; Whiteley 2011). Active buffering, however, comprises higher energetic costs and thus could require higher metabolic rates or energy reallocation from other important processes (Wood et al. 2008). Uncompensated acidosis can lead to a metabolic depression (i.e. the down-regulation of energy demands and the aerobic metabolism) and thus, in the long-term, to reduced growth rates or other important fitness related traits (Michaelidis et al. 2005; Pörtner et al. 2004).

OA directly affects calcification processes in marine organisms due to a decrease in CO_3^{2-} concentrations and a lowering of the $CaCO_3$ saturation state in the seawater (Fabry 2008). To a certain extent, many calcifying organisms exert a control over biomineralization processes involving passive and active ion transport to calcification compartments, which are isolated from ambient seawater (Weiner and Dove 2003). However, reduced calcification rates under simulated OA have been observed in many marine species (reviewed in e.g. Fabry et al. 2008; Doney et al. 2009). Due to their importance for the global carbon cycle, special attention has been dedicated to planktonic calcifiers such as coccolithophores or foraminifera (e.g. Riebesell et al. 2000; Iglesias-Rodriguez et al. 2008; Lombard et al. 2010). Commercial benthic calcifiers such as echinoderms or molluscs have further been objects for extensive OA related research over the last decade (e.g. Gazeau et al. 2007; Thomsen and Melzner 2010; Dupont et al. 2010; Lebrato et al. 2010). Corals have been demonstrated to be among the most sensitive groups to OA (Hoegh-Guldberg et al. 2007; Doney et al. 2009). In a laboratory study, Fine and Tchernov (2007) showed that the scleractinian coral *Oculina patagonica* completely lost its skeleton when grown in acidified water (pH 7.4) for an extended period although with the ability to recover calcareous structures under normal pH conditions. While most data so far stem from short-term studies, the dominant reef-building cold-water coral *Lophelia pertusa* has for example been shown to be capable to acclimatize to long-term exposure to OA conditions (Form and Riebesell 2012). Further, the coccolithophore *Emiliania huxleyi* even showed evolutionary adaptation to OA stress by the selection over 500 generations (Lohbeck et al. 2012), which sheds new light on the real impacts OA might have in the future. Nevertheless, in a study on several marine calcifiers, Ries et al. (2009) demonstrated a remarkable diversity of the responses of marine calcifiers to mid-term OA.

Although there is limited empirical data available, early life stages are considered to be more sensitive to environmental stressors (with mortality rates of up to over 90% during their early days of life) than juvenile or adult stages (Gosselin and Qian 1999; Kurihara 2008). Many early life-history stages still lack specialized regulatory tissues (Hu et al. 2011) or immune response systems and have to further undergo dramatic transitions between different larval stages as well as the metamorphosis from the larvae to juvenile (Walther et al. 2010; Kurihara 2008). The metamorphosis to the early juvenile includes dramatic morphological changes, which also can involve shifts in feeding behaviour and food sources (Pechenik 1999). In most cases, marine invertebrates undergo a transition from the pelagic to the benthic environment when developing from one life stage to another, during which they are exposed to different abiotic (e.g. temperature, salinity, pH, light) and biotic (e.g. predation, fouling, competition) stressors (Pechenik 1999). Larval responses to certain stressors can directly determine the overall recruitment success but also cause subsequent effects on the performance of later stages or juveniles (Miron et al. 2000; Pechenik et al. 1998; Thiyagarajan et al. 2005; Emlet and Sadro 2006).

Studying OA effects on the early life stages of organisms is becoming more and more common in the OA community. Nevertheless, to date, most studies have investigated adult stages of marine invertebrates (Kroeker et al. 2010 and e.g. Gazeau et al. 2007; Ries et al. 2009; Thomsen et al. 2010; Appelhans et al. accepted in Marine Ecology-Progress Series). A first study highlighting the importance of investigating the response of larvae to OA showed that echinoderm (*Ophiothrix fragilis*) larvae e.g. suffered from 100% mortality under reduction in 0.2 pH units (Dupont et al. 2008). Further examples show that OA has the ability to dramatically hamper the performance of marine invertebrate larvae (reviewed in e.g. Kurihara 2008; Dupont et al. 2010; Kroeker et al. 2010). Nevertheless, a noticeably high proportion of studies demonstrate OA effects to be rather sub-lethal to larvae merely extending single larval stages or the entire larval phase than affecting overall survival (e.g. Stumpp et al. 2011).

While calcifying organisms are typically more affected by OA than non-calcifiers (Kroeker et al. 2010) highly mobile organisms such as teleost fish (e.g. Checkley et al. 2009; Munday et al. 2009), cephalopods (e.g. Gutowska et al. 2010a, b; Hu et al. 2011) or crustaceans (e.g. McDonald et al. 2009; Fehsenfeld et al. 2011; Appelhans et al. accepted in Marine Ecology-Progress Series) appear to be generally more tolerant of OA (discussed in Pörtner 2008; Melzner et al. 2009; Ries et al. 2009; Kroeker et al. 2010). Some species have been demonstrated to even express higher calcification rates under OA conditions (Wood et al. 2008; McDonald et al. 2009; Gutowska et al. 2010a). Beyond these first general estimates of OA impacts on single marine species, predictions for OA based changes in future marine ecosystems are difficult. A first comprehensive community-based mesocosm study, demonstrated a shift in the community towards a lower diversity under

OA with molluscs being most affected while annelids or arthropods were rather tolerant of OA (Hale et al. 2011).

The responses to OA however, seem to be highly species-specific (Kroeker et al. 2010) and acclimation (e.g. Form and Riebesell 2012) or adaptation (e.g. Lohbeck et al. 2012) are important issues to be considered for future predictions. Thus, estimates from short-term laboratory experiments on single species are difficult. Good opportunities to study future OA effects are regions, which are already naturally acidified. E.g. investigations of species communities in the vicinity of volcanic CO_2 vents in Ischia, Italy show an inverse correlation between the degree of CO_2 mediated acidification and the occurrence of calcifying organisms such as calcareous algae, sea urchins, limpets or barnacles (Hall-Spencer et al. 2008; Fig. 1.7). In regions where calcifying organisms were absent, sea grass species or non-calcareous algae became dominant, representing a strong shift in the ecosystem community under acidified conditions. One major issue regarding these natural CO_2 vents is, however, the large pH variability around the discussed mean values of 8.14, 7.76 and 6.47, casting some doubt on these specific zones for assessing the effect of future OA (Riebesell 2008; Kerrison et al. 2011). Furthermore, these naturally acidified areas are rather small scaled and gene drift from adjacent areas might hamper local populations to adapt to OA. Thus, naturally large scale acidified areas could be a more suitable choice to examine possible future community changes in an acidified ocean (Thomsen et al. 2010).

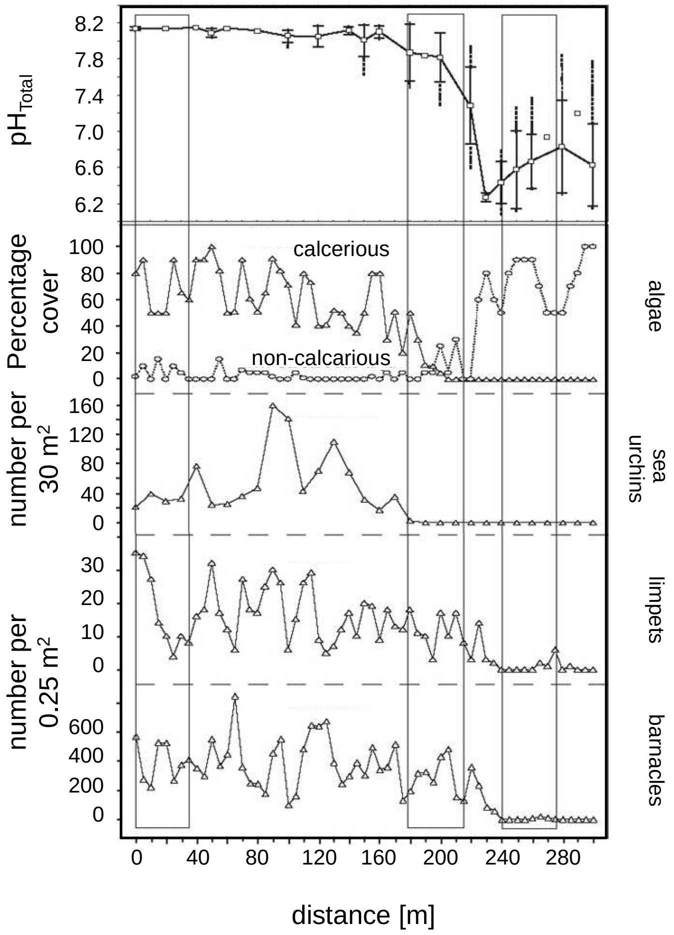

Figure 1.7 Variation in pH (mean pH ± s.d.: cross bars; ranges: dotted lines), percentage cover of calcareous (triangles) and non-calcareous algae (circles) and abundance of sea urchins, limpets and barnacles at CO_2 vents south of Ischia, Italy (from Hall-Spencer et al. 2008).

1.3. Warming and Eutrophication

Stress effects such as OA will, particularly in times of global change, not act alone but in combination with additional stressors such as warming or desalination (Harley et al. 2006). Multiple stressors can act additively, antagonistically or synergistically and it will be an important task to investigate multiple stressor effects on organisms' performance, in order to reliably predict future ecosystem changes (Przeslawski et al. 2005; Wahl et al. 2011).

Observations since the 1960's show that the average temperature of the global ocean has increased to depths of 3000 m and that to date the ocean has absorbed more than 80% of the heat added to the global climate system (IPCC 2007). Global sea surface temperatures have thus risen in the past century by 0.4 to 0.8 °C and are expected to further increase (IPCC 2001; 2007). Warming affects the abundance or distribution of single species and has the potential to alter pelagic and benthic marine ecosystems (Harley et al. 2006; Rosenzweig et al. 2008). Warming and OA have been reported to act in synergy with regard to marine ectotherms (O'Donnell et al. 2009; Pörtner 2008; Pörtner and Farrell 2008; Parker et al. 2010; Pörtner 2010). Walther et al. (2010) showed e.g. that severe OA (3000 µatm) narrows the thermal tolerance window of adult crabs (*Hyas araneus*). In contrast, recent studies by Brennand et al. (2010) and Waldbusser et al. (2011) show that warming can also mitigate negative effects of OA on the growth of sea urchin larvae and on the calcification of oysters.

An obvious ecosystem response to eutrophication is the greening of the oceans due to algal growth in direct response to nutrient enrichment. The most common effects of eutrophication on marine ecosystems are perceived as increases in the abundance of algae and aquatic plants but also as the occurrence of increasingly severe toxic phytoplankton blooms (Smith et al. 1999). Thus, eutrophication can lead to far-reaching ecosystem shifts with substantial economic effects (Smith et al. 1999). In coral reefs, increased nutrient inputs can lead to shifts from coral-dominated to algal-dominated communities (e.g. Chazottes et al. 2002). A further serious threat from eutrophication is the decrease in dissolved oxygen levels in bottom waters, which arises when planktonic algae die and lead to increasing microbial respiration in bottom waters (Diaz and Rosenberg 2008 and references therein). Declines in dissolved oxygen due to eutrophication was first observed in many coastal habitats worldwide in the late 1950's after starting the extensive use of industrially produced nitrogen fertilizer. Since the mid of the 20th century, the number of dead zones worldwide has approximately doubled each decade (reviewed in Diaz and Rosenberg 2008). Although, large differences in species responses to OA have been observed between eutrophic and oligotrophic coastal habitats (e.g. Hall-Spencer et al. 2008; Thomsen et al. 2010), the interactive effects of energy supply and OA have however, rarely been tested in empirical studies to date. Thomsen et al. (under review in Nature Climate Change) investigated the impacts of energy supply and OA on the mussel *Mytilus edulis* and concluded that available energy was the main driver affecting calcification and growth.

1.4. Study Areas

The Skagerrak and Kattegat with an overall area of about 61 000 km^2 connects the North Sea with the Baltic Sea (Fig. 1.8). The Skagerrak reaches a depth to 700 meters at the Norwegian Trench. The Baltic Sea is a semi-enclosed marginal sea with an overall area of about 387 000 km^2 and an average depth of only about 55 m. The Landsort Deep represents the deepest part with a maximum of 459 m. Due to limited exchange of water masses with the North Sea and strong terrestrial runoff as well as net precipitation, the Baltic Sea exhibits a gradual salinity decline along a west to north-east gradient. Salinity values range between 30 in the Skagerrak to 20 in the Kattegat and reaches down to 2 in the Gulf of Finland or the Gulf of Bothnia. Salinity levels also vary with depth. The boundary between less dense and less saline surface waters and saltier deep waters is known as the halocline, which lies at a depth between 60 and 80 m. In summer, an additional thermocline divides surface waters into a wind-mixed surface layer (down to 10–25 m) and a deeper, denser and colder layer (down to the seafloor or the halocline; HELCOM 2003). This strong stratification inhibits the vertical exchange of water masses and thus of dissolved or gaseous compounds.

In the Kiel Fjord e.g. (located in the Kiel Bight in the western Baltic Sea), stratification during summer can lead to hypoxic conditions in bottom water (Thomsen et al. 2010), mainly driven by the heterotrophic degradation of sedimented plankton blooms (Hansen et al. 1999; Broecker 2003). This process is accompanied by an increase in $CO_{2(aq)}$ and a decrease in the seawater pH. Wind-driven upwelling events of saline and cold deep-water masses can lead to acidified conditions in shallow marine habitats (Vincent Saderne pers. commun.). Thus, pCO$_2$ and pH levels in the Kiel Fjord already today periodically exceed values predicted to occur in the open ocean by the end of this century (Thomsen et al. 2010). While annual mean pCO$_2$ values of 969 µatm and an annual mean pH of 7.76 have been measured (Thomsen 2012), the pCO$_2$ in shallow waters can even fluctuate between 100 and 2700 µatm with pH fluctuations of 7.3 to 8.4 over the year (Jörn Thomsen pers. commun.). This particularity of coastal zone chemistry may not only anticipate future OA as predicted in current models (e.g. Caldeira and Wickett 2005; Orr et al. 2005), but may also further amplify acidification driven by atmospheric CO$_2$ uptake. With a doubling of current atmospheric CO$_2$ concentrations, periodical seawater pCO$_2$ values of even 4000 µatm can be expected in the Western Baltic Sea (Melzner et al. 2012). Seasonal hypoxia is a natural process in this area but increasing eutrophication further enhances productivity and thus amplifies oxygen depletion and temporal acidification (Babenerd 1991; HELCOM 2003, 2009; Conley et al. 2007). Hypoxic zones are spreading (Diaz and Rosenberg 2008; Zillén et al. 2008) and large-scale upwelling of acidified water has been reported in other regions (e.g. Feely et al. 2008, 2010) and, thus, estuaries and coastal marine habitats such as the Baltic Sea are more susceptible to changes in pH than open ocean waters.

The Baltic Sea, as an enclosed marginal sea, makes it highly sensitive to eutrophication. Eutrophication is a phenomenon of the recent past and since the 1950s, the surplus nitrogen due to agriculture has approximately tripled within the Baltic Sea region. Eutrophication has been demonstrated to change phytoplankton biomass and species composition as well as macrofauna biomass (manly macrozoobenthos above the halocline), which already increased five-fold from the 1920's–30's however to the 1990s (The BACC author team 2008). Since the mid-1980's however, eutrophication has stopped to increase in the Baltic region (Rheinheimer 1998; The BACC author team 2008). But even though there have been

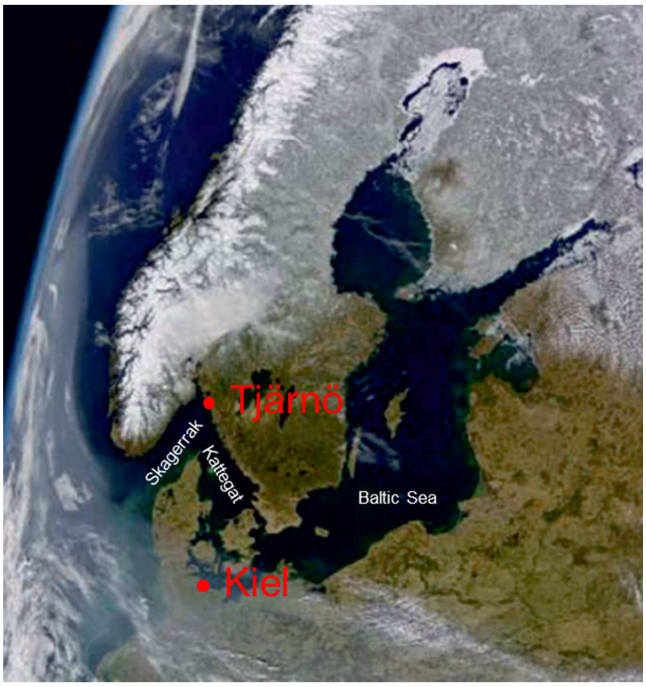

Figure 1.8 The Skagerrak, the Kattegat and the Baltic Sea as seen from the SeaWiFS satellite (NASA/Goddard Space Flight Center, GeoEye) on 1 April 2004 (from The BACC author team 2008).

dramatic changes in agricultural policy and practices since the 1990’s, the quality of the Baltic Sea has not significantly improved to date (The BACC author team 2008). The Kiel Fjord as a habitat with additionally substantial river inflow, thus, it still represents a highly eutrophied habitat (Nausch et al. 2011). Eutrophication has already effected species composition and abundance within the Baltic ecosystem and due to the characteristics of the Baltic Sea with limited exchange of water masses with the open ocean, there will likely not be considerable changes in the eutrophication status over the next decades.

Projected future warming within the Baltic Sea basin generally exceeds the predictions from global climate model simulations by up to 50% with temperatures expected to increase within the Baltic Sea by 3 to 5 °C within the next 100 years (Meier 2006; HELCOM 2003, 2007; The BACC author team 2008) with likely threats to local marine communities (Harley et al. 2006; Rosenzweig et al. 2008).

Due to the strong salinity gradient from the Skagerrak to the Gulf of Bothnia, the Baltic Sea is characterized by a transition from marine to few “true” brackish species and further to freshwater species. Thus, compared to other aquatic ecosystems only relatively few species occur in the more brackish ecosystems of the Baltic Sea. This limited number of species as well as the appearance of single key-species makes the Baltic Sea a unique ecosystem (HELCOM 2003). Salinity in the Kiel Fjord (located in the Kiel Bight in the western Baltic Sea) ranges between 10 and 20 with an annual mean of about 15 in shallow waters. In this habitat, the two benthic filter feeders *Mytilus edulis* and *Amphibalanus improvisus* dominate large areas of the hard-bottom benthic ecosystem (Enderlein and Wahl 2004; Dürr and Wahl 2004). The two dominant consumers *Carcinus maenas* and *Asterias rubens* predate on mussels and barnacles (Enderlein and Wahl 2004, Reusch and Chapman 1997). Future changes on the interactions between these few key-species could have remarkable impacts on the entire ecosystem.

The less frequent occurrences of oxygen minimum zones, as well as the close connection of the Skagerrak to the Atlantic ocean suggests that the Tjärnö Archipelago would be less variable in carbonate chemistry compared to the Kiel fjord and Baltic Sea. Although long-term data sets are not available at this point, this has been reported in earlier studies (Larsson 2010) and was also confirmed by Havenhand et al. (unpublished data). Seawater pH in shallow habitats, as documented to date, can vary in the range of 0.3 pH units on a daily basis, but it rarely falls below pH 8.1, at least during the period investigated (April to Mai; Larsson 2010). The Tjärnö archipelago has a tidal range of 0.3 m and consists of rocky shores and islands, frequently interrupted by shallow, soft-bottom bays and fjords (Jonsson et al. 2004). The salinity around Tjärnö is around 25 and thus, in average, about salinity 10 units higher than in the Kiel Fjord. The Skagerrak is furthermore characterized by much higher species diversity and thus more complex networks of interactions between species. The disappearance of a single key-species might therefore have less severe effects on community structures since niches can be easily overtaken by other species.

1.5. Barnacles as Model Organisms

Globally barnacles are ecologically important ecosystem components (Foster 1987; Leppäkoski and Olenin 2000) and are abundant within the species poor Baltic Sea (Berntsson and Jonsson 2003). Published studies suggest that environmental shifts affecting barnacle growth such as warming and food availability (Stanford and Menge 2001; Skinner et al. 2007) or OA (Findlay et al. 2010c) will likely affect recruitment and population dynamics of barnacles. Since barnacles are commercially relevant biofoulers and adequate model organisms to investigate the responses to environmental stressors in different life stages, they have been widely used in experimental studies over many years (reviewed in Briand 2009).

The barnacle species *Amphibalanus (Balanus) improvisus* (Darwin 1854) (Pitombo 2004) can be found in shallow, tidal or non-tidal areas worldwide in oceanic and brackish waters (Foster 1987; Olenin and Leppäkoski 1999) but this species has also been found in depths of up to 50 meters. In the Baltic Sea *A. improvisus* was first recorded in 1844 (Leppäkoski and Olenin 2000), ten years before the species formally was described by Darwin in 1854. *A. improvisus* originates from North America and was most likely introduced to the Baltic region through hull fouling on ships (Leppäkoski and Olenin 2000). Today this

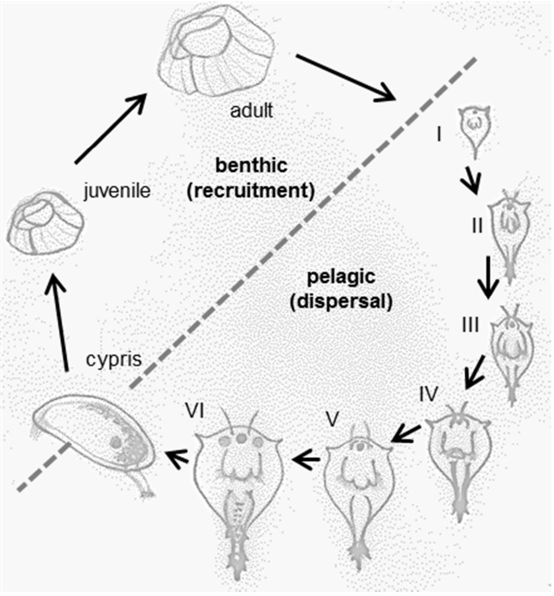

Figure 1.9 Life cycle of *Amphibalanus (Balanus) improvisus* illustrating 6 pelagic nauplius stages, the non-feeding cypris stage and the benthic juvenile as well as adult stage (modified from Jones and Crisp 1954 and http://origin-ars.sciencedirect.com).

species occurs from the Gulf of Bothnia and Gulf of Finland to the Swedish west coast (Jansson 1994).

A. improvisus mainly recruits during summer and early autumn but shows occasional recruitment throughout the year (Berntsson and Jonsson 2003; Thomsen et al. 2010). While most barnacles are cross-fertilizing hermaphrodites, *A. improvisus* has been shown to sometimes self-fertilize (Furman and Yule 1990). Fertilization takes place internally and egg masses develop within the mantle cavity. Single barnacle individuals thereby release about 1000 to 10 000 larvae. Eggs hatch as free-swimming stage-I nauplius larvae and develop into stage-II nauplii within 3-4 h (Thiyagarajan et al. 2003). Nauplii mainly feed on diatom algae (Nasrolahi et al. 2007) and pass through six larval stages before they metamorphose into a non-feeding cypris larva (Jones and Crisp 1954), which settles and metamorphoses to the juvenile barnacle (post-larva; Fig. 1.9). Barnacle cyprids are highly specialized to search for suitable settlement substrates and are therefore equipped with highly complex sensory organs (Anil et al. 2010). The cypris larva lacks any feeding organs and is thus depended on the energy reserve, which it accumulated during the nauplius phase and stored in fat cells until settlement (Thiyagarajan et al. 2002, 2005). Settlement of cyprids depends on parameters such as substrate characteristics, turbidity, biofilms or the presence of conspecifics (Maki et al. 1988; Dineen and Hines 1992; Berntsson et al. 2004; Larsson and Jonsson 2006; Nasrolahi 2007). Juveniles start feeding on phyto- and zooplankton about 4 to 5 days after settlement and grow rabidly to the adult barnacles reaching maturity within a few months (Thiyagarajan et al. 2003; McDonald et al. 2009). Barnacles moult every 2 to 3 days and with age, the relation of the shell to body size naturally increases leaving space for egg development (Costlow and Bookhout 1957).

Juvenile and adult barnacles consist of six plates represented in Fig. 1.10. The rostrum and the rostrolaterals are in this species merged to one single plate. The plates consist of $CaCO_3$ and organic components with organic material merely representing a proportion of up to 3% and the $CaCO_3$ in *A. improvisus* as in most barnacle species is in form of the more stable calcite (Barnes et al. 1976; Bourget 1987; Findlay et al. 2010a). The plates are covered by an

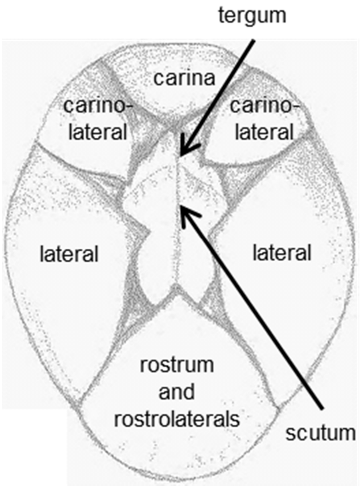

Figure 1.10 Illustration of shell plates of *Amphibalanus (Balanus) improvisus* (modified from Rainbow 1984).

epicuticula while longitudinal canals go through the plates (Rainbow 1984). Biomineralization in barnacles takes place between the inner side of the shell plates and the mantel epithelium (Fig. 1.11) and is assumed to be processed by specialized cells present in the outer mantel epithelium (Rainbow 1984; Anderson 1994; Gohad et al. 2009). The basal plate attaches the barnacle to the substrate due to the secretion of proteinaceous cement by the cement gland (Rainbow 1984).

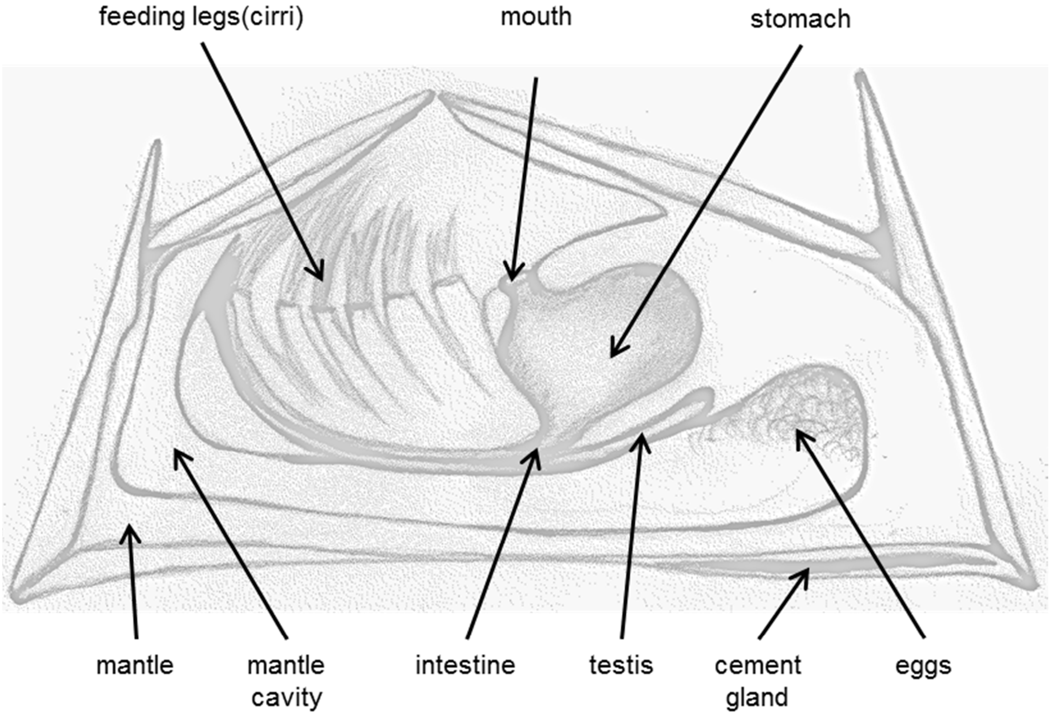

Figure 1.11 Semi-diagrammatic sagittal section of *Amphibalanus (Balanus) improvisus* (modified from Jones and Crisp 1954 and http://origin-ars.sciencedirect.com).

Following the terminology of Kinne (1964), *A. improvisus* is a euryhaline species, i.e. being able to successfully perform in water conditions ranging from fresh water to fully marine seawater (Luther 1987; Fyhn 1976; Leppäkoski and Olenin 2000; pers. obs.). This species is furthermore capable of tolerating low oxygen concentrations and even strong habitat pollution (Leppäkoski and Olenin 2000). Gohad et al. (2009) describes all life stages of *A. amphitrite* to possess chloride ion rich epithelia with a large potential for active ion transport. Although most barnacle species are osmoconformers, *A. improvisus* is thought to be partly capable of hyperosmotic regulation of the haemolymph and the cell volume in waters below 500 mOsm (Fyhn 1976). The haemolymph osmolality (measure of the moles of solute per kilogram) in the experiments by Fyhn (1976) never reached below

100 mOsm. There are to date, however, no studies available illustrating the haemolymph pH of *A. improvisus*. In the gooseneck barnacle *Pollicipes polymerus* a haemolymph pH of down to 7 was measured in submerged animals being possibly similar to that in *A. improvisus* (Fyhn 1976 and references therein).

In the study by Powers (1920) it was suggested that the abundance and the average size of species such as barnacles are dependent on pH values present in the surrounding environment. Empirical studies on barnacles' performance under OA are rare (presented in Table 1.1) but recently experiments have been conducted with the barnacles *Elminius modestus* (Findlay et al. 2010a) *Semibalanus balanoides* and (Findlay et al. 2009, 2010a, b) and *A. amphitrite* (McDonald et al. 2009). Juvenile to adult *E. modestus* suffered from reduced growth rates under OA (pH 7.7, 1100 µatm pCO_2) while survival and shell composition were not affected (Findlay et al. 2010a). Survival and shell-calcium content of juvenile *S. balanoides* (south UK population) were significantly reduced under OA (pH 7.7, 1100 µatm pCO_2; Findlay et al. 2009) and under the combination of warming (+4 °C) and OA (pH 7.7, 1100µatm pCO_2) while growth was not significantly affected (Findlay et al. 2010a). Growth and survival of juvenile *S. balanoides* (Spitsbergen population) were negatively affected under OA (pH 7.7 and 7.4, 1100 and 2400 µatm pCO_2) while the shell composition remained unaffected (Findlay et al. 2010b). Juvenile *A. amphitrite* exhibited increased growth and calcification in basal regions of the shell plates and thus stronger attachment to the substrate while older regions of the plates showed reduced shell strength under OA (pH 7.4; McDonald et al. 2009). Fertilization in barnacles has not been investigated to date but the embryonic development of *S. balanoides* was significantly delayed under OA (pH 7.7 and 7.4, 1100 and 2400 µatm pCO_2; Findlay et al. 2009). Larval condition, cyprid size, cyprid attachment and metamorphosis as well as juvenile to adult growth and egg production of *A. amphitrite* were not affected by OA (pH 7.4; McDonald et al. 2009).

Temperature strongly affects barnacles' larval development with a generally enhanced development under elevated temperatures (e.g. Anil and Kurian 1996; Qiu and Qian 1999; Thiyagarajan et al. 2003; Desai and Anil 2004; Nasrolahi et al. 2012). In juvenile and adult barnacles however, increased temperature rather induces negative responses with reduced growth rates or survival (Findlay et al. 2010a, b). Barnacles such as *A. improvisus* can be particularly abundant in eutrophied harbours where its abundance can be one or two orders of magnitude greater than in natural environments (Vuorinen et al. 1986). Thus, barnacles seem to generally benefit from high energy supply and might as the consequence be competitive with other benthic filter feeders such as the mussel *Mytilus edulis* under these conditions.

Table 1.1: Overview of the experiments available on the performance of barnacles under OA stress.

species	*p*CO_2 (pH) levels [µatm, pH units]	start	origin	barnacle collection	duration	reference
Semibalanus balanoides	12°C: 346, 922 (8.07, 7.70)	23rd November 2007	south UK	-	15 weeks	Findlay et al. 2009
	investigated parameters:	post-larval survival and shell mineralogy (Ca, Mg, Ca:Mg ratio), embryonic development				
	significant effects under OA:	22% lower post-larval survival, changes in the post-larval mineral structure of shells (Mg decrease), slower embryonic development rates				
	non-significant trends under OA:	-				
	15°C: 409, 1132 (8.05, 7.73) 20°C: 423, 1109 (8.07, 7.71)	30th April 2007	south UK	1 week old post-larvae from the field	4 weeks	Findlay et al. 2010a
	investigated parameters:	post-larval survival, growth and shell Ca content				
	significant effects under OA:	-				
	non-significant trends under OA:	reduced survival by 10-25%, reduced growth by 73% under 15°C, slightly reduced shell Ca content				
	5°C: 352, 1086, 2429 (8.12, 7.68, 7.35) 9°C: 343, 1060, 2448 (8.14, 7.71, 7.36)	4th August 2008	Spitsbergen	1 week old post-larvae from the field	3 weeks	Findlay et al. 2010b
	investigated parameters:	post-larval survival, growth and shell mineralogy (Ca, Mg, Ca:Mg ratio)				
	significant effects under OA:	reduced post-larval growth by 33/72% (in 5°C) and by 61/83% (in 9°C)				
	non-significant trends under OA:					
Elminius modestus	15°C: 413, 1076 (7.96, 7.73) 20°C: 412, 1075 (7.98, 7.73)	13th August 2007	south UK	1 week old post-larvae from the field	4 weeks	Findlay et al. 2010a
	investigated parameters:	post-larval survival, growth and shell Ca content				
	significant effects under OA:	reduced growth rates by 43% (in 20°C)				
	non-significant trends under OA:	slightly reduced shell Ca content				
Amphibalanus amphitrite	25 - 28°C: - (8.2, 7.4)	August 2008	North Carolina, USA	freshly hatched nauplii from the lab	1 week (larvae) 11 weeks (post-larvae)	McDonald et al. 2009
	investigated parameters:	larval development, cyprid size, cyprid settlement, juvenile to adult growth, egg production, adhesion strength, shell strength				
	significant effects under OA:	19% increased adhesion strength, increased CaCO3 levels in basal plates, decreased shell strength				
	non-significant trends under OA:	increased growth rates				

1.6. Objectives and Hypotheses

Although most OA research on barnacles and on other marine species to date has focussed on single rather than multiple stress effects (e.g. McDonald et al. 2009) and on single rather than consecutive life-history stages (e.g. Findlay et al. 2010a, b) it is of high importance to consider, especially in the fate of global change, multiple stress scenarios in ecological experiments on the response of the entire life cycle of a species to these predicted changes.

In the present study I hypothesized that:

H1: OA will affect the performance of different life-history stages of the barnacle *A. improvisus*.

H2: Early life-history stages of the barnacle *A. improvisus* are more vulnerable to OA than adult stages.

H3: Predicted warming will enhance OA effects on the barnacle *A. improvisus*.

H4: Food availability will affect the capacity of *A. improvisus* to cope with future OA.

H5: Stress effects will be carried over from parents to their F1 generation.

H6: Barnacle populations from fluctuating $p\mathrm{CO}_2$ environments are more tolerant to OA than barnacles from more stable $p\mathrm{CO}_2$ habitats.

2. General Materials and Methods

Six main experiments were conducted between July 2008 and December 2011 summarized in Table. 2.1. The first experiment was conducted on the development of nauplius and cypris larvae from the Kiel Fjord (54°19.5‘N, 10°09.0‘E) under different temperature and OA conditions (Table. 2.1: E-1). A similar but slightly simplified setup of the experiment was used to investigate the development of nauplius larvae from the Tjärnö Archipelago (58°52.5‘N, 11°08.1‘E) under different OA conditions (Table. 2.1: E-2). Juvenile barnacles from Kiel Fjord were studied under different temperature and OA conditions in two subsequent experiments in a “batch culture” and a “water-flow through” setup (Table. 2.1: E-3+4). To investigate juvenile *A. improvisus* response to OA under different food regimes on, two parallel experiments were conducted with individuals from the Kiel Fjord and the Tjärnö Archipelago (Table. 2.1: E-5+6).

Table 2.1: Overview of the conducted experiments and experimental parameters investigated.

experiment	time frame	experimental location	origin of animals	life stages investigated	mean CO_2 or pH perturbation levels]pH	temperature [°C]	additional stressors	carbonate system measurements	experimental duration	measured response variables	publication
1	Jul-Sep 2008	Kiel	Kiel Fjord	nauplii and cyprids	400, 1250, 3250	20	temperature: -8, +7 °C	pH_{NBS}, C_T, A_T	8 (nauplii) and 4 (cyprids) weeks	survival, larval duration, development	I
2	Oct 2009	Tjärnö	Tjärnö Archipelago	nauplii	8.0, 7.8, 7.6	25		pH_{NBS}	1 week	survival, larval duration, development, larval stage proportion, larval size	II
3	May-Sep 2010	Kiel	Kiel Fjord	juveniles	700, 1000, 2140	20	temperature: +4°C	pH_{NBS}, pH_T, C_T	8 weeks	survival, growth, DW, CI, shell strength	III
4	Oct-Jan 2010/2011	Kiel	Kiel Fjord	juveniles to adults	620, 1030, 1930, 2870	20		pH_{NBS}, pH_T, C_T	12 weeks	survival, growth, DW, CI, shell strength	III
5	May-Dec 2011	Kiel	Kiel Fjord	juveniles to adults	477, 966 , 2720	20	food limitation: -80%	pH_{NBS}, pH_T, C_T	20 weeks	survival, growth, DW, AW, CI, moulting, reproduction, shell strength, offspring fitness	IV
6	Aug-Sep 2011	Tjärnö	Tjärnö Archipelago	juveniles	562, 1123, 3211	20	food limitation: -80%	pH_{NBS}, pH_T, pCO_2	5 weeks	survival, growth, DW, AW, CI	IV

A barnacle culture was installed with the ability to continuously obtain *A. improvisus* nauplius larvae for the different experiments (Fig. 2.1). For this purpose, adult barnacles, either attached to stones or to grey PVC panels (Fig. 2.2), were collected from the Kiel Fjord and placed into 15 l aquaria with a constant flow-through of filtered (sand filter) seawater at a constant temperature of 20 °C. The adult barnacles were fed with brine shrimps (*Artemia salina*). Whenever larvae were needed, the entire system as well as the barnacles itself were cleaned from *Artemia* (life and dead individuals as well as cysts) and other particles meanwhile the *A. improvisus* individuals were exposed to desiccation for two hours. This procedure imposed a certain stress to the barnacles releasing larvae after re-submersion in the flow-through water system (Fig. 2.1). Larvae were then obtained by sieving overflowing seawater through 90µm plankton nets overnight (Fig. 2.1). Existing barnacle culture facilities in Tjärnö, Sweden provided nauplius larvae for the Tjärnö experiments.

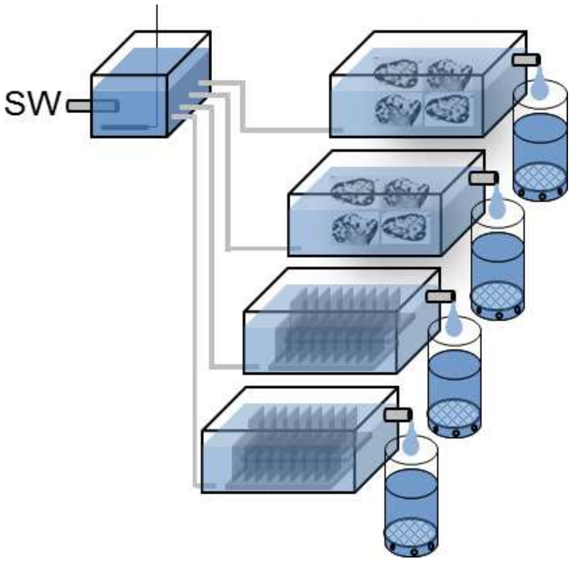

Figure 2.1 Barnacle (*Amphibalanus improvisus*) culture facilities at the GEOMAR, Kiel.

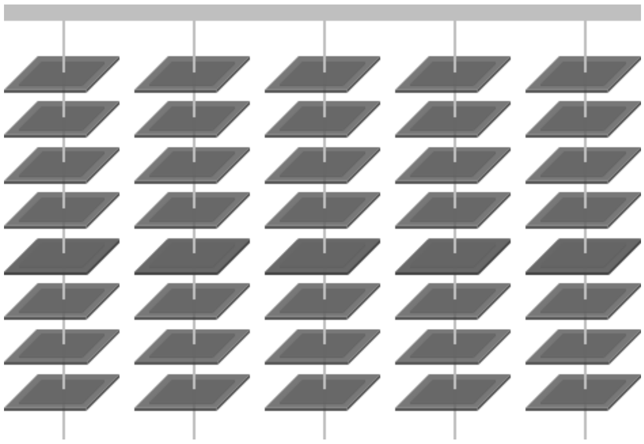

Figure 2.2 Collection of juvenile barnacles on PVC panels within the Kiel Fjord or the Tjärnö Archipelago.

To obtain freshly settled barnacles for the “juvenile” experiments, settlement panels were exposed to natural colonisation in the habitats of interest for two weeks in 1.5 meter water depth (Fig. 2.2). The panels to be used in the corresponding experiments were covered by a wider grey PVC panel to prevent detritus and settlement of barnacles and other organisms on the upwards-facing side of the panel. This procedure also prevented settlement of algae due to the reduced light availability underneath the panels.

In the first experiment (E-1), nauplius as well as cypris larvae were investigated under different temperature and OA stress. Therefore three temperature (12, 20 and 27 °C) and three $p\mathrm{CO}_2$ treatments (400, 1250 and 3250 µatm) were applied in a fully crossed experimental design with 8 replicates each (for details see Table 2.1; Fig. 2.3 and publication I). The experiments were run in 6-well plates, filled with 8 ml filtered (0.2 µm) seawater in equilibrium with an overlying atmosphere of different $p\mathrm{CO}_2$ concentrations. This was achieved by placing the 6-well plates into sealed 2 l plastic aquaria, which were filled with 1.5 l seawater and aerated with pressurized air of ambient or enriched $p\mathrm{CO}_2$ concentrations. A central gas mixing facility at the GEOMAR, Kiel (Linde Gas®, HTK Hamburg, Germany) provided and air flow with fixed $p\mathrm{CO}_2$ concentrations of 1400 and 4000 ppm. Ambient untreated air was used to aerate the “control” aquaria. The 2 l aquaria were additionally placed in temperature baths (100 l glass aquaria) at the treatment temperatures (Aqua Medic®, Germany). The water within the water baths was constantly mixed (common aquarium pump) to avoid any temperature gradients within the baths.

The experiment on nauplius larvae from the Tjärnö Archipelago (E-2) comprised a similar but slightly reduced setup system wherein water $p\mathrm{CO}_2$ was manipulated by adding pure gaseous CO_2 using computerized pH controllers (NBS scale, resolution: 0.01 units, Aqua Medic GmbH, Germany; for details see Table 2.1 and publication II). Temperatures of 25 °C were chosen for reasons of comparability with already existing data sets.

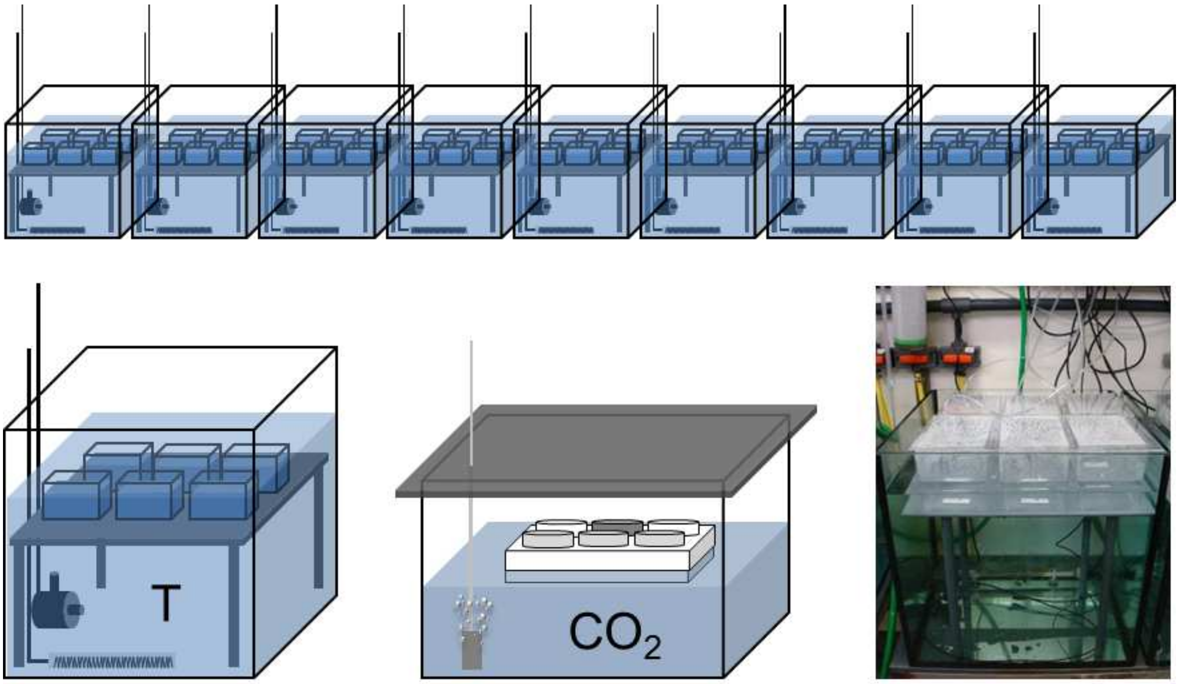

Figure 2.3 Scheme of the experimental setup of the Kiel larval (nauplii and cyprids) experiment: Nine 100 l glass aquaria were set to one of the three experimental temperatures. Each contained six sealed 2 l plastic aquaria. 6-well plates were placed into the 2 l aquaria floating on filtered seawater, which was aerated with pressurized air of fixed $p\mathrm{CO}_2$ concentrations. Single wells of the 6-well plates were filled with filtered seawater + 20 nauplii or 10 cyprids (dark grey well) or filtered seawater only, left for water replacements (light grey well).

In the third experiment (E-3), juvenile barnacles were reared in 1 l plastic beakers under temperature and OA stress (for details see Table 2.1; Fig. 2.4 and publication III). The plastic beakers were aerated with pressurized ambient air or air of fixed (see above) pCO_2 concentrations of 1120 and 4000 ppm. The 1 l plastic beakers were additionally placed in temperature baths (see above) set at 20 and 24 °C. Each treatment was replicated 8 times. Two PVC panels were placed in one beaker and swapped between beakers every second day while the water in the remaining beakers was exchanged and left to equilibrate to the target temperature and pCO_2.

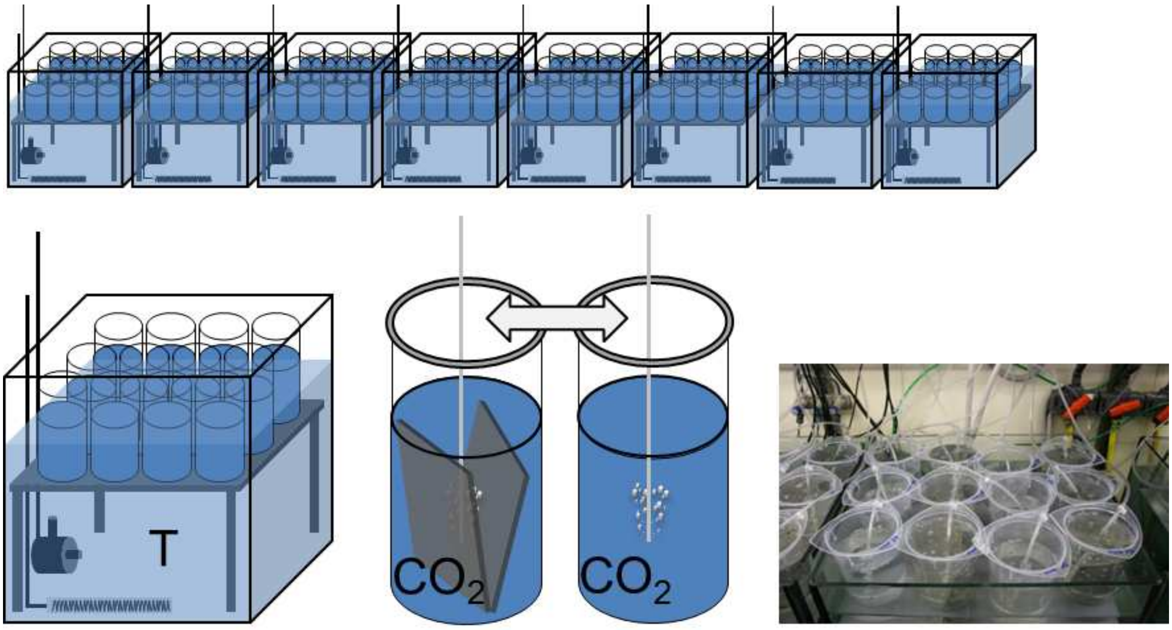

Figure 2.4 Scheme of the experimental setup of the first Kiel juvenile experiment: eight 100 l glass aquaria set to one of the three temperatures and constantly mixed with an aquarium pump contained twelve 1 l plastic beakers each. The plastic beakers were aerated with pressurized air of fixed pCO_2 concentrations. The panels were swapped between beakers every second day while the water in the remaining beakers was exchanged and left to equilibrate to the target temperature and pCO_2.

In the fourth experiment (E-4), juvenile barnacles were reared in 3.7 l plastic aquaria under OA stress at 20 °C (for details see Table 2.1; Fig. 2.5 and publication III). The plastic aquaria were aerated with pressurized ambient air or air of fixed (see above) pCO_2 concentrations of 1120, 2400 and 4000 ppm. Each treatment was replicated 5 times. Filtered (sand filter) flow-through seawater from the Kiel Fjord was pumped into a storage tank of 250 l (aerated and tempered) and further pumped into a header tank of 60 l (set at 20 °C; Aqua Medic®, Germany) from which the water ran into the 3.7 l sealed plastic aquaria. Two panels were screwed together (barnacle individuals facing to the inside) and placed into the plastic aquaria.

The fifth and the sixth experiment (E-5+6) were conducted in a similar experimental setup as the fourth experiment (for details see Table 2.1; Fig. 2.5 and publication IV) in Kiel as well as in Tjärnö. The plastic aquaria were aerated with pressurized ambient air or air of fixed pCO_2 concentrations of 1120 and 4000 ppm in Kiel (see above). The experiment in Tjärnö comprised a similar setup system wherein water pCO_2 was manipulated by adding pure gaseous CO_2 using computerized pH controllers (NBS scale, resolution: 0.01 units, Aqua Medic GmbH, Germany, IKS® Computer Systeme GmbH, Germany) at nominally 1000 and 3000 µatm pCO_2 (for details see Table 2.5 and publication IV).

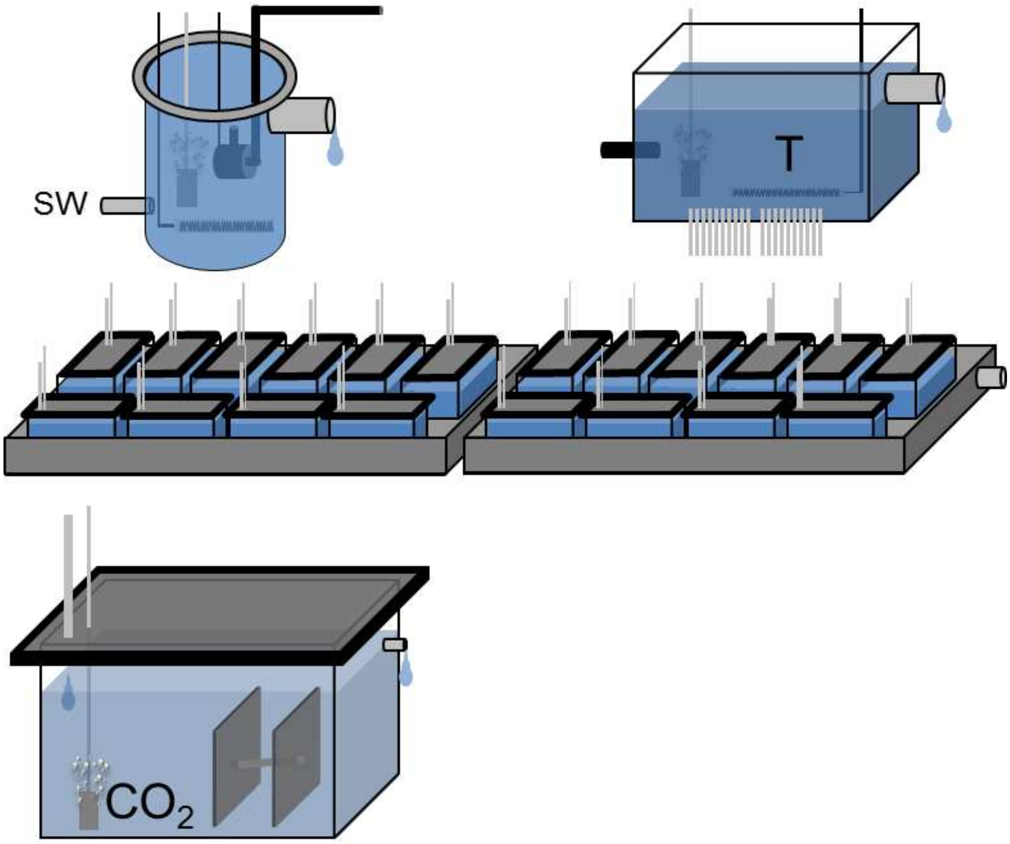

Figure 2.5 Scheme of the experimental setup of the second Kiel juvenile experiment equipped with a flow-through water system: sand filtered seawater (SW) was pumped into a storage tank of 250 l (aerated and tempered) and further pumped into a header tank of 60 l (set at 20°C) from which the water run into 3.7 l sealed plastic aquaria which were directly aerated with pressurized air of fixed pCO_2 concentrations. Two panels were screwed together and placed into the plastic aquaria (barnacle individuals facing to the inside).

3. Publications

All experiments were designed and conducted by myself or with support of the co-authors and all manuscripts were written by myself, and revised together with the co-authors.

I. Pansch C, Nasrolahi A, Appelhans YS, Wahl M (2012) Impacts of ocean warming and acidification on the larval development of the barnacle *Amphibalanus improvisus*. Journal of Experimental Marine Biology and Ecology, 420–421, 48-55.

Statement of authorship: CP and MW designed the study, CP and AN collected the data, CP analysed the data and wrote the manuscript and all authors contributed substantially to revisions

II. Pansch C, Schlegel P, Wahl M, Havenhand JN (prepared for submission in Marine Ecology-Progress Series) Larval development of the barnacle *Amphibalanus improvisus* in an acidified ocean.

Statement of authorship: CP, PS, MW and JNH designed the study, CP and PS collected the data, CP and PS analysed the data and wrote the manuscript and all authors contributed substantially to revisions

III. Pansch C, Nasrolahi A, Appelhans YS, Wahl M (under review in Marine Biology) Ocean warming and ocean acidification - impacts on juvenile *Amphibalanus improvisus*.

Statement of authorship: CP and MW designed the study, CP and AN collected the data, CP analysed the data and wrote the manuscript and all authors contributed substantially to revisions

IV. Pansch C, Schaub I, Havenhand JN, Wahl M (prepared for submission in Global Change Biology) Do habitat traits and food availability modulate the impact of ocean acidification on juvenile *Amphibalanus improvisus*?

Statement of authorship: CP and IS contributed equally to this work. CP, IS and MW designed the study, CP and IS collected and analysed the data and wrote the manuscript and all authors contributed substantially to revisions

Publication I – Larval development of the Kiel population

Impacts of ocean warming and acidification on the larval development of the barnacle Amphibalanus improvisus.

Impacts of ocean warming and acidification on the larval development of the barnacle *Amphibalanus improvisus*.

Christian Pansch*, Ali Nasrolahi, Yasmin Shirin Appelhans, Martin Wahl

[1]Department of Marine Ecology – GEOMAR | Helmholtz Centre for Ocean Research, 24105 Kiel, Germany

Statement of authorship: CP and MW designed the study, CP and AN collected the data, CP analysed the data and wrote the manuscript and all authors contributed substantially to revisions

Corresponding author*:

Tel: +49 431 600 4084
Fax: +49 431 600 1671
Email: cpansch@geomar.de

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Abstract

The world's oceans are warming and becoming more acidic. Both stressors, singly or in combination, impact marine species, and ensuing effects might be particularly serious for early life stages. To date most studies have focused on ocean acidification (OA) effects in fully marine environments, while little attention has been devoted to more variable coastal ecosystems, such as the Western Baltic Sea. Since natural spatial and temporal variability of environmental conditions such as salinity, temperature or $p\text{CO}_2$ impose more complex stresses upon organisms inhabiting these habitats, species can be expected to be more tolerant to OA (or warming) than fully marine taxa. We present data on the variability of salinity, temperature and pH within the Kiel Fjord and on the responses of the barnacle *Amphibalanus improvisus* from this habitat to simulated warming and OA during its early development. Nauplii and cyprids were exposed to different temperature (12, 20 and 27 °C) and $p\text{CO}_2$ (nominally 400, 1250 and 3250 µatm) treatments for 8 and 4 weeks, respectively. Survival, larval duration and settlement success were monitored. Warming affected larval responses more strongly than OA. Increased temperatures favoured survival and development of nauplii but decreased survival of cyprids. OA had no effect upon survival of nauplii but enhanced their development at low (12 °C) and high (27 °C) temperatures. In contrast, at the intermediate temperature (20 °C), nauplii were not affected even by 3250 µatm $p\text{CO}_2$. None of the treatments significantly affected settlement success of cyprids. These experiments show a remarkable tolerance of *A. improvisus* larvae to 1250 µatm $p\text{CO}_2$, the level of OA predicted for the end of the century.

Introduction

Persistent anthropogenic CO_2 emissions have led to increased CO_2 uptake by the oceans (IPCC 2007; Sabine et al. 2004). Dissolved CO_2 decreases the pH of the ocean, a phenomenon called ocean acidification (OA) (Feely et al. 2004; Orr et al. 2005). Since the industrial revolution, oceanic pH has dropped by nearly 0.1 units (Caldeira and Wickett 2005; Orr et al. 2005). Modelling predicts a drop in seawater pH by 0.3 to 0.4 units by the end of the century and a drop of 0.7 to 0.8 by 2300 (reaching $p\text{CO}_2$ values of about 1000 and 2000 µatm, respectively; Caldeira and Wickett 2005; IPCC 2007; Orr et al. 2005). OA could directly affect vital biological processes and indirectly alter fundamental structures of pelagic and benthic marine ecosystems (Harley et al. 2006; Riebesell 2008).

Over the past 10 years, a large number of studies have reported on impacts of OA on marine invertebrates (reviewed in Doney et al. 2009; Dupont et al. 2010a; Fabry et al. 2008). However, although early life stages are thought to be more sensitive to environmental stressors (Dupont et al. 2010a, b; Gosselin and Qian 1997; Kurihara 2008), only few studies have investigated OA effects during early ontogenetic development. In brittlestar (*Ophiothrix fragilis*) larvae Dupont et al. (2008) observed dramatic effects (100% mortality) under a 0.2 unit decrease in pH. Less dramatic or no effects of OA were observed in other studies (e.g. oyster sperm motility: Havenhand and Schlegel 2009; copepod performance and reproduction: Kurihara and Ishimatsu 2008; growth of sea star larvae: Dupont et al. 2010c).

The first comprehensive meta-analyses detected predominantly negative but highly variable responses of invertebrates to OA (Dupont et al. 2010a, b; Hendriks et al. 2010; Hendriks and Duarte 2010; Kroeker et al. 2010). Calcifying organisms are typically more affected by OA than non-calcifiers (Hendriks et al. 2010; Kroeker et al. 2010), and highly mobile organisms such as cephalopods or crustaceans seem to be less sensitive to OA (Gutowska et al. 2010; Kroeker et al. 2010; Melzner et al. 2009; Pörtner 2008; Ries et al. 2009). Responses to OA seem to be, however, highly species specific or habitat dependent. Polar species, which are naturally exposed to a lower saturation of $CaCO_3$ and more variation in pH, are arguably better adapted to OA than their counterparts from the tropics (Andersson et al. 2008; Clark et al. 2009; see also Dupont et al. 2010a). Dupont et al. (2010a) go even further and debate that echinoderm larvae, which are released into seawater with a naturally high variability in pH, can cope better with predicted OA than larvae normally experiencing more stable pH conditions (see also Byrne et al. 2009; Dupont et al. 2008).

In the Baltic Sea, stratification during summer can lead to hypoxic conditions in bottom water, mainly driven by the heterotrophic degradation of sedimented plankton blooms (Diaz and Rosenberg 2008; Shim et al. 2007; Wootton et al. 2008). This process is accompanied by an increase in dissolved CO_2. Wind-driven upwelling events of saline and cold deep water masses can, thus, further lead to acidified conditions in shallow marine habitats. River discharges of acidic water can further exacerbate acidification in estuarine and coastal habitats (Salisbury et al. 2008). Thus, $p\mathrm{CO}_2$ levels in the Kiel Fjord even today periodically exceed values predicted to occur in the open ocean by the end of this century (Thomsen et al. 2010). Hypoxic zones are spreading (Diaz and Rosenberg 2008) and large-scale upwelling of acidified water has been reported in other regions (e.g. Feely et al. 2008, 2010). These particularities of coastal zone chemistry may not only anticipate future OA as predicted in current models (e.g. Caldeira and Wickett 2005; Orr et al. 2005), they may also further amplify acidification driven by atmospheric CO_2 uptake (Borges and Gypens 2010; Feely et al. 2010; Miller et al. 2009; Salisbury et al. 2008). With a doubling of current atmospheric CO_2 concentrations, periodical seawater pCO_2 values of even 4000 µatm can be expected in the Western Baltic (Melzner et al. 2012).

While in locally acidified areas such as volcanic CO_2 vents (Hall-Spencer et al. 2008) adaptation to acidified conditions may be hindered by gene flow from adjacent non-impacted areas, adaptation seems possible when entire regions such as the Baltic Sea feature (temporarily) acidified conditions. Populations adapted to pH fluctuations in these coastal habitats may, thus, exhibit reduced sensitivity to predicted acidification.

OA will not act alone but rather in synchrony with other predicted environmental shifts such as warming. In the Baltic Sea annual mean water temperatures are expected to increase by 1.9 – 3.2 °C within the next 100 years (The BACC author team 2008; Meier 2006). Warming impacts the abundance or distribution of single species and has the potential to alter pelagic and benthic marine ecosystems (Harley et al. 2006; Rosenzweig et al. 2008). Warming and OA have been reported to act in synergy with regard to marine ectotherms (O’Donnell et al. 2009; Pörtner 2008; Pörtner and Farrell 2008; Parker et al. 2010; Pörtner 2010). Walther et al. (2010) showed that severe OA (3000 µatm) narrows the thermal tolerance window of adult crabs (*Hyas araneus*). In contrast, recent studies by Brennand et al. (2010) and Waldbusser et al. (2011) show that warming can mitigate negative effects of OA on growth of sea urchin (*Tripneustes gratilla*) larvae and on calcification in oysters.

The impact of OA on early life stages of barnacles has received little attention so far. Larval stages of the barnacle *Amphibalanus amphitrite* seem not affected by OA (McDonald et al. 2009), while the embryonic development of *Semibalanus balanoides* was slowed under elevated pCO_2 (Findley et al. 2009). An empirical model by Findlay et al. (2010c), suggests that combined effects of warming and OA will have much stronger effects than single factors would have on the barnacle *Semibalanus improvisus*. While several reports show moderate warming to be beneficial for barnacle larval development (e.g. Anil et al. 1995; Qiu and Qian 1999; Thiyagarajan et al. 2003, Nasrolahi et al. 2012), to our knowledge, no studies have investigated the combined effects of warming and OA upon the early development of barnacles. Indeed, most studies focused on single rather than multiple stressor effects (e.g. Dupont et al. 2008; McDonald et al. 2009) or on single rather than consecutive life stages of organisms (e.g. Findley et al. 2010a). In the course of global change, however, multiple stressors will be common and the fate of species will be determined by the most sensitive life stages exposed to various stressors. Additionally, most OA studies have focused on fully marine environments (or open ocean species in lab cultures) and little attention was given to more variable near-shore ecosystems. To better understand the possible impact of future OA, we need to incorporate multi-stress scenarios, different life stages and fluctuating habitats into our investigations. In this study, we investigated the sensitivity of two early life stages (nauplius and cypris larvae) of *Amphibalanus improvisus* from the highly fluctuating Kiel Fjord to combinations of predicted future warming and OA.

Materials and methods

A. improvisus can be found in shallow, tidal or atidal areas worldwide in oceanic and brackish waters (Foster 1987). In the Western Baltic, *A. improvisus* is by far the most common barnacle species. It mainly recruits during summer and early autumn but shows occasional recruitment throughout the year (Berntsson and Jonsson 2003; present study). While most barnacles are cross-fertilizing hermaphrodites, *A. improvisus* has been shown to sometimes self-fertilize (Furman and Yule 1990). Fertilization takes place internally and egg masses develop within the mantle cavity. Eggs hatch as free-swimming stage-I nauplius larvae and develop to stage-II nauplii within 3-4 h (Thiyagarajan et al. 2003). Nauplii pass through 6 stages before they metamorphose into a non-feeding cypris larva (Jones and Crisp 1954), which settles and metamorphoses to the juvenile barnacle (post-larva).

Abiotic conditions and barnacle settlement within the Kiel Fjord

Salinity, temperature and pH_{NBS} measurements were done on monthly cruises between April 2009 and May 2010 at 4 different stations within the inner Kiel Fjord (54°19.7‘N, 10°09.6‘E; 54°19.9‘N, 10°09.9‘E; 54°19.9‘N, 10°09.3‘E; and 54°19.7‘N, 10°09.1‘E) and the GEOMAR pier (54°19.5‘N, 10°09.0‘E) using a CTD 60 (Sea and Sun Technology® GmbH). Mean values were calculated for a water depth of 1 to 1.5 m. To determine seasonal settlement patterns of macrofouling (including barnacles), settlement panels were exposed to the natural colonizer pool at the GEOMAR pier at a depth of 1 m monthly between March 2005 and October 2010. Settlement panels were made of grey PVC (5 × 5 cm) manually roughed using grain-60 sandpaper to facilitate attachment. After one-month of exposure, settlement panels were gently rinsed to remove unattached organisms. Foulers at the downwards-facing side of the settlement panels were identified to the genus or species level and "% cover" of all species (including *A. improvisus*) was estimated for each month (N = 6).

Warming and acidification effects on nauplius and cypris larvae

Experiments were conducted in constant temperature rooms of the GEOMAR, Kiel, Germany in late summer 2009. Adult specimens of the barnacle *A. improvisus* (attached to stones) were collected from a subtidal zone in the "Schwentine" estuary within the Kiel Fjord (54°19.7‘N, 10°11.1‘E) when the water had a temperature of 20 °C and a salinity of 10. The barnacles were kept in the laboratory in a water flow-through system at 20 °C and a salinity of 15 for five days and fed daily with ample brine shrimps (*Artemia salina*). To obtain fresh larvae, we gently cleaned the adult barnacles under a stream of tap water to remove any *A. salina* and exposed them to air for 2 h. This procedure leads to the release of larvae after re-submersion. We collected nauplius larvae by sieving (90 µm mesh) overflowing seawater over night.

The temperature treatments of 12, 20 and 27 °C and $p\mathrm{CO}_2$ treatments of 400, 1250 and 3250 µatm (for details see Table 1) were chosen because they correspond to conditions in the Kiel Fjord today (Thomsen et al, 2010, Fig. 1a). Furthermore, they include the shifts predicted for the coming decades and centuries (IPCC, 2007, The BACC author team, 2008), and they are supposed to represent – at least in the case of OA – no, intermediate and high stress, respectively.

All experiments on early life stages of *A. improvisus* were run in 6-well plates (Greiner bio-one®, Smurfit Kappa GmbH, Neuburg-Germany), filled with 8 ml natural filtered (0.2 µm) seawater in equilibrium with an overlying atmosphere of different $p\mathrm{CO}_2$ concentrations (Fig. 2; see also Egilsdottir et al. 2009). This was achieved by placing the 6-well plates into sealed 2 l plastic aquaria, which were filled with 1.5 l filtered seawater (0.2 µm) and aerated with pressurized air of fixed $p\mathrm{CO}_2$ concentrations at ambient, 1400 and 4000 ppm (mean ± s.d.: ambient air, 1396 ± 34 or 3967 ± 58 ppm $p\mathrm{CO}_2$). The aquaria were additionally placed in temperature baths at treatment temperatures of 12, 20 and 27 °C (Fig. 2). Within each 6-well plate, we used one well for rearing of nauplii or cyprids (experimental well; Fig. 2: dark grey well), while the other wells were used to independently equilibrate filtered seawater (0.2 µm) to the respective target temperature and $p\text{CO}_2$ conditions (equilibration well; Fig. 2: light grey wells). The water in each experimental well was replaced every other day (nauplius experiment) or every third day (cypris experiment) by preconditioned seawater from the equilibration wells (Fig. 2).

Pilot experiments have shown that a 2 day period is sufficient to equilibrate seawater within the 6-well plates with the respective $p\text{CO}_2$ of the surrounding atmosphere. Additional data on the effects of nauplii respiration or algal photosynthesis/respiration in a comparable system (with similar larval and food concentrations as mentioned above) showed the pH within the experimental units to be little affected by nauplii but rather by the algal food. Therefore, pH was logged (IKS® Computer Systeme GmbH, Germany) in sealed 2 l plastic aquaria and in modified 50 ml tubes (substitute for wells) filled with pre-equilibrated filtered seawater (0.2 µm) in equilibrium with an overlying atmosphere at fixed $p\text{CO}_2$ concentrations (ambient and 4000 ppm) at 20 °C every 10 min over 24 h (as equivalent to 24 h post water exchange in the experiments; N=3). Mean pH values (mean ± s.d.) in the sealed 2 l plastic aquaria were 8.14 ± 0.01 (aerated with ambient air) and 7.24 ± 0.01 (aerated with air $p\text{CO}_2$ values of 4000 ppm). Tubes which were placed within the atmosphere of the aerated aquaria showed pH values of 8.09 ± 0.01 (ambient) and 7.25 ± 0.01 (4000 ppm) when they were filled with filtered seawater only and pH values of 8.12 ± 0.02 (ambient) and 7.26 ± 0.03 (4000 ppm) when nauplii were added to the tubes. Tubes containing nauplii and food showed elevated pH conditions of 8.23 ± 0.05 (ambient) and 7.42 ± 0.07 (4000 ppm). Thus, the $p\text{CO}_2$ treatment values chosen for the main experiment cover a sufficiently wide range with biological effects to be smaller than the treatment effects.

Salinity (WTW® Cond 340i equipped with a TetraCon® 325 electrode), pH and temperature (WTW® 330i pH meter equipped with a Sentix-81® pH electrode; NBS scale) measurements were conducted every 10 days by pooling water samples from the equilibration wells (Fig. 2: light grey wells). Additional water samples for C_T (dissolved inorganic carbon) and A_T (total alkalinity) measurements were taken thrice during the experiment by pooling water from different equilibration wells as mentioned above. C_T (dissolved inorganic carbon) was determined coulometrically (SOMMA® autoanalyser) and A_T was analysed by potentiometric titration (VINDTA® autoanalyser). Measurements were corrected using DICKSON seawater standard as reference (Dickson et al. 2003). All other parameters (pH_T, $p\text{CO}_2$, Ω_{Ca} and Ω_{Ar}) were calculated using the CO_2SYS macro

(Pierrot et al. 2006) with dissociation constants (K_1 and K_2) according to Millero (2006) using $KHSO_4$ dissociation constant after Dickson (1990).

Calculated mean pCO_2 values across the different temperatures (i.e. 400, 1250 and 3250 µatm pCO_2) will indicate the acidification treatment levels in the following. We conducted two parallel experiments to assess the sensitivity of nauplius and cypris stages of *A. improvisus* in response to OA under different temperatures.

Nauplius development

Nauplius larvae were reared for two months at three temperature and pCO_2 levels (mentioned above) in a fully crossed design. 20 stage-II nauplii were added to one well containing 8 ml filtered seawater (0.2 µm) at ambient air pCO_2 and 20 °C in each of 54 6-well plates. These 6-well plates were then distributed among 54 2 l aquaria, each maintained at one of the 9 possible temperature x pCO_2 combinations. Each of these treatment combinations was replicated 6 times. The targeted temperature and pCO_2 conditions were allowed to slowly build up in the Nauplii-containing well over several hours.

The water in the wells was replaced every other day by filtered seawater (0.2 µm) that was preconditioned to the respective target temperature and pCO_2 (see Fig. 2 and description above). Larvae were fed daily with marine diatoms (1:1 mixture of *Chaetoceros calcitrans* and *Skeletonema costatum*, 1-1.5 x 10^5 cells per ml; Nasrolahi et al. 2007). In order to minimize pH variability due to food addition, the algal mix taken from the algal culture was carefully centrifuged at 3000 rpm (50 ml falcon tubes) and re-suspended in seawater with the respective target pCO_2 before being added to the wells. The experiment was run for 57 days under continuous light (~25.3 µmol photons $m^{-2}s^{-1}$) to minimize mortality and deposition of algal cells (Thiyagarajan et al. 2003). Nauplius survival, occurrence of cyprids and settlement were assessed every 2 days using a binocular microscope (WILD® M3C Heerbrugg, Switzerland). We defined “larval duration” as the time (days) in which 50% of the surviving nauplii had metamorphosed to the cypris stage. If nauplii had neither changed to cyprids nor died after 57 days (this occurred twice in the 12 °C and 3250 µatm treatment), we conservatively set the larval duration at 57 d.

Cypris settlement

In this experiment we assessed the response of cypris larvae to the same temperature x $p\mathrm{CO}_2$ regimes as in the nauplius experiment. Stage-II nauplii were reared to the cypris stage in aerated (ambient air $p\mathrm{CO}_2$) 20 l buckets at 20 °C under continuous light (using the same diatom algae and food concentrations as mentioned above). The obtained cyprids were then distributed to 6-well plates with 10 cyprids per well in 8 ml filtered seawater (0.2 µm) and exposed to the different temperature and $p\mathrm{CO}_2$ conditions. Water was exchanged every 3 days as described above. Each treatment was replicated 6 times. Survival and settlement success were monitored every 3 days over a period of 29 d.

Statistical analysis

In the present study, a fully crossed two-factorial design was used with temperature (3 levels) and $p\mathrm{CO}_2$ (3 levels) as the fixed factors. We tested for normality with the Shapiro-Wilk’s W-test and homogeneity of variances was assessed using Levene’s test. Larval duration data were log transformed to meet parametric assumptions, while all other data were square-root arcsine transformed before performing statistical tests. When assumptions of normality and homogeneity of variances were met, a two-factorial analysis of variance (ANOVA) was applied while we used permutational analysis of variance (PERMANOVA) when one of the assumptions was violated (Anderson et al. 2008; Clarke and Gorley, 2006). To analyse the effects of temperature and $p\mathrm{CO}_2$ on the final survival and development of nauplii, PERMANOVA was applied using “Bray-Curtis similarity” or “Euclidean distance” matrices with 9999 permutations. The effects of $p\mathrm{CO}_2$ and temperature on the larval duration as well as on the final survival and settlement of cyprids were analysed using a two-factorial ANOVA. We used PERMANOVA pair-wise tests or Fisher’s least significant difference (LSD) tests for multiple comparisons following PERMANOVA or ANOVA, respectively. All statistics were performed using Primer 6 (version 6.1.12) with PERMANOVA+ (version 1.0.2) and STATISTICA 8.0 (Stat- Soft, Inc., USA).

Results

In the field, barnacles are exposed to extremely variable conditions in the course of a year. Between April 2009 and May 2010, salinity of the Kiel Fjord varied between 8.5 and 19.2 (Fig. 1a) and temperature varied between -0.3 and 19.7 °C (Fig. 1a). Seawater

pH_{NBS} fluctuated between 7.51 and 8.33 reaching lowest values during late summer and early autumn (Fig. 1a). *A. improvisus* settled throughout the year with the highest mean settlement between May and September (Fig. 1b).

The above field data show that at least the 2 lower of our temperature and $p\text{CO}_2$ treatment levels are not uncommon in the habitat of the barnacles. In our experiment, there was a significant effect of temperature, but not of $p\text{CO}_2$ on the final survival of nauplii after 57 days (Fig. 3a-d; Table 2). Both factors did not interact significantly (Fig. 3a-d; Table 2). Mean survival in the different treatments ranged from 4 to 25% (Fig. 3d). Survival was reduced by a factor of 3 at 12 °C compared to survival at the two higher temperatures (means over $p\text{CO}_2$ treatments: 12 °C = 7%, 20 °C = 22%, and 27 °C = 20%; Fig. 3d).

There was a significant effect of temperature but not of $p\text{CO}_2$ on the final development (i.e. proportion of nauplii metamorphosed to cyprids and post-larvae) after 57 days, while a significant interaction between temperature and $p\text{CO}_2$ was observed (Fig. 3a-d; Table 2). With increasing temperature, larvae developed faster with the first cyprids appearing on day 8 in 27 °C and on day 10 in 20 °C (Fig. 3a-c). In 12 °C water, first cyprids were observed on day 21 (Fig. 3a) and only 8, 9 and 3% of the initial 20 nauplii had developed to the cypris stage after 57 days under 400, 1250 and 3250 µatm, respectively (Fig. 3d).

Temperature but not $p\text{CO}_2$ significantly affected the larval duration (time of development from stage-II nauplii to cyprids; Fig 4; Table 3). Temperature and $p\text{CO}_2$ interacted significantly (Fig 4; Table 3). The longest mean larval duration of 44 days was observed in 12 °C and 3250 µatm (Fig. 4) where even after 57 days some live nauplii had not yet changed to cyprids. The fastest mean larval development of 11 days was observed under 27 °C and 400 µatm (Fig. 4).

There was a significant effect of temperature and $p\text{CO}_2$ on the final survival of cyprids (originating from nauplii, which were raised under ambient conditions of 20 °C and ambient $p\text{CO}_2$) after 29 days (Fig. 5a-d; Table 4). Both factors did not interact significantly (Fig. 5a-d; Table 4). Overall, the final survival of cyprids was reduced in higher temperatures (means over $p\text{CO}_2$ treatments: 12 °C = 81%, 20 °C = 59%, and 27 °C = 29%; Fig. 5d). At 27 °C, final mean survival of cyprids was reduced by 14% under control $p\text{CO}_2$ compared to the 1250 and 3250 µatm $p\text{CO}_2$ treatments (Fig. 5c).

There was no significant effect of temperature or $p\text{CO}_2$ on the final settlement of cyprids after 29 days nor was there a significant interaction between the two factors (Fig.

5a-d; Table 4). While the highest final mean settlement of 40% was observed in 20 °C water under 3250 µatm pCO_2, all other treatment combinations showed lower settlement between 16 and 28% (Fig. 4d).

No morphological abnormalities, neither in nauplii nor in cyprids or freshly settled barnacles were detected by microscopic examination (under any of the treatment combinations). We observed a strong temporal variability in development of nauplius larvae even within a single experimental unit (i.e. treatment combination). This, however, was not statistically tested.

Discussion

Acid-base disturbances, caused by pCO_2 stress (hypercapnia) can cause metabolic depression in marine invertebrates (Pörtner et al. 2004), which possibly led to a delay in larval development of *A. improvisus* in the present study. Metabolic depression is, however, a transient response to OA. Thus, more likely, nauplius larvae under high pCO_2 might have re-allocated energy to maintenance of basic metabolic rates having less energy available for growth and development (Thomsen et al. 2010). The finding that this developmental delay was particularly strong at 12 °C could either reflect a direct combined effect of both stressors (low temperature and high pCO_2) or an indirect effect with a slower development at low temperatures (Anil et al. 2001; Nasrolahi et al. 2012; Thiyagarajan et al. 2003) prolonging the exposure of a given life stage to high pCO_2 (see discussion in McDonald et al. 2009).

The observed OA impacts at the high temperature may have a different reason. Mean larval duration at 27 °C was extended under moderate and severe OA (1250 and 3250 µatm pCO_2). High temperature enhances swimming activity (Yule 1984) and thus the metabolic scope of nauplii. If the available energy or the aerobic capacity of nauplii were limited, a trade-off between swimming and development may have led to the developmental delay at 1250 and 3250 µatm pCO_2.

Warming and OA antagonistically affected survival of cyprids in the present study. Enhanced mortality under elevated temperature was not surprising, since survival of the non-feeding cypris stage ultimately depends on the amount of stored energy and elevated temperatures increase metabolic demands and the likelihood of depleted energy stores in cyprids (Thiyagarajan et al. 2002, 2005). If elevated pCO_2 led to a metabolic depression in cyprids, lowered energy demands could explain the observed longer survival of this non-feeding larval stage under high pCO_2 in the present study. There were neither significant temperature effects nor were there pCO_2 effects on the settlement of cyprids. Dupont et al. (2010c) obtained similar results for lecithotrophic echinoderm larvae and juveniles of the sea star *Crossaster papposus* and suggested that species with lecithotrophic life stages are less dependent on exogenous sources, such as phytoplankton (and its digestion) and may, thus, be better adapted to future OA compared to species with planktotrophic life stages.

When larval duration is prolonged under OA (present study), *A. improvisus* nauplii, in a high-CO_2 ocean, might be exposed to predation for a longer time period. Using model calculations, Kennedy (1996) postulated a reduction of 89% in successful recruitment of *Crassostrea virginica* when metamorphosis was delayed by 25% (from 20 to 25 days). Thus, OA might have severe indirect consequences for barnacles with possible implications on the ecosystem level. In addition, prolongation of the nauplius phase might lead to a mismatch of the larvae with their phytoplankton prey (Svensson et al. 2005). On the other hand, the predicted increase in seawater temperature, (IPCC 2007; Meier 2006) will likely accelerate nauplii development (present study; Anil et al. 2001; Nasrolahi et al. 2012; Thiyagarajan et al. 2003) and, thus, may buffer OA effects. Such results were observed in sea urchin larvae (*Tripneustes gratilla*) and oysters (*Crassostrea virginica*), where higher temperatures mitigated negative effects of OA (Brennand et al. 2010; Waldbusser et al. 2011). In the present study, in contrast to nauplii, warming negatively impacted cypris survival while OA counteracted these negative effects. Thus, the impact of warming and OA depends on whether these factors act singly or in combination as well as on the larval stage considered. Predictions on the fate of barnacles are further complicated by equally complex but mostly unstudied impacts of warming and OA on species interacting with barnacle nauplii such as its predators (e.g. Javidpour et al. 2009). Warming and OA could also affect prey performance (e.g. algal photosynthesis) in different ways affecting CO_2 and oxygen levels in surrounding seawater of nauplii larvae with possible effects on the experimental results.

While in the present study OA-effects on *A. improvisus* larvae were observed at the high and low temperature treatments, OA had no effect at all at 20 °C. This pattern might be explained by the ability of organisms to better tolerate even severe OA stress when being kept in their optimal thermal window range (Pörtner 2008, Pörtner and Farrell 2008; Pörtner 2010). It should also be stressed that only the most severe OA level applied herein (3250 µatm pCO_2) had occasional effects, whereas the OA conditions as predicted by the end of this century (1250 µatm pCO_2) in most cases did not affect *A. improvisus* larvae in the present study. This apparent insensitivity to moderate OA may reflect an acclimatization or adaptation of benthic organisms in the Western Baltic to generally strong natural $p\mathrm{CO}_2$ fluctuations and an annual mean (± s.d.) $p\mathrm{CO}_2$ of 705 ± 461 µatm within the inner Kiel Fjord (Thomsen et al. 2010). Water samples occasionally taken at the sampling site (54°19.7‘N, 10°11.1‘E) even showed $p\mathrm{CO}_2$ levels of up to 1700 µatm ($\Omega_{\mathrm{Calcite}} = 0.62$; $\Omega_{\mathrm{Aragonite}} = 1.09$) during summer 2009 (Pansch, unpublished data). Given their present wide tolerance and the possibility to adapt to shifting environmental conditions over many generations, barnacles (*A. improvisus*) from the Western Baltic Sea might be able to overcome OA as predicted by the end of this century. Supporting this, Parker et al. (2011) show selectively bred lines of the estuarine oyster *Saccostrea glomerata* to be more resilient to OA than wild populations. It remains to be seen, however, whether *A. improvisus* populations from more stable habitats are less tolerant.

Longer data sets for the Kiel Fjord show that *A. improvisus* tolerates wide fluctuations of salinity (9 – 22), temperature (-0.5 – 25 °C) and also pH (7.5 – 8.1; Fig. 1a; Thomsen et al. 2010; Wahl et al. 2010). Interestingly, the major release of larvae and thus, development, settlement and first intense calcification in *A. improvisus* occurs during early summer when pH is lowest (Fig. 1 a, b; Thomsen et al. 2010). In the Kiel Fjord, *A. improvisus* is also found in stands of the brown macroalga *Fucus* spp. where 2500 µatm $p\mathrm{CO}_2$ (pH 7.4) can be measured (Vincent Saderne pers. commun.). Another barnacle species, *Chthamalus stellatus*, was shown to survive and grow at extremely low mean pH of 6.6 in the vicinity of volcanic CO_2 vents in Ischia, Italy (Hall-Spencer et al. 2008).

Even if warming x OA effects are small during the larval stages of *A. improvisus*, carry-over effects on the metamorphosis or post-larval growth in barnacles (Thiyagarajan et al. 2002, 2005) could exist and neglecting them may lead to under-estimation of the real impacts of OA (see also discussion in Dupont et al. 2010b). Negative effects of OA on post-larvae have been previously reported (Findley et al. 2009, 2010a, b; McDonald et al. 2009).

Relevant abiotic variables such as salinity temperature or pH vary spatially across the species’ distributional range and temporally during the lifespan of individuals, more strongly than predicted for climate change effects over the coming decades (e.g. Fig. 1; Thomsen et al. 2010; Wootton et al. 2008). Thus species such as the barnacle *A. improvisus* with a wide distribution and its populations from strongly fluctuating habitats (e.g. benthic coastal and/or marginal habitats), may be able to better cope with climate change than organisms from more stable habitats (Dupont et al. 2010a; Parker et al. 2011; Miller et al. 2009).

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Tables

Table 1: Temperature, salinity, pH_{NBS} (N = 6), dissolved inorganic carbon (C_T) and total alkalinity (A_T) (N = 3) measurements (N = 3) as well as calculated pH_{NBS}, pCO_2, $\Omega_{Calcite}$and $\Omega_{Aragonite}$values (mean ± s.d.).

			Seawater measurements			Calculations from C_T and A_T			
T [°C]	Air pCO_2 [ppm]	Sal	pH_{NBS}	C_T [µmol kg^{-1}]	A_T[µmol kg-1]	pH_{NBS}	pCO_2 [µatm]	$\Omega_{Calcite}$	$\Omega_{Aragonite}$
12.1 ± 0.2	ambient	15.1 ± 0.5	8.04 ± 0.05	1960.66 ± 28.4	2057.30 ± 32.2	8.21 ± 0.03	405.85 ± 22.5	2.37 ± 0.13	1.39 ± 0.07
	1400		7.67 ± 0.03	2069.02 ± 36.9	2056.47 ± 33.6	7.75 ± 0.01	1230.77 ± 49.41	0.89 ± 0.02	0.52 ± 0.02
	4000		7.26 ± 0.05	2205.06 ± 61.2	2071.11 ± 62.10	7.33 ± 0.02	3320.95 ± 58.61	0.35 ± 0.02	0.21 ± 0.01
19.6 ± 0.4	ambient	16.1 ± 0.5	8.11 ± 0.08	1948.18 ± 6.4	2086.59 ± 8.3	8.22 ± 0.04	413.54 ± 37.3	3.25 ± 0.21	1.95 ± 0.12
	1400		7.72 ± 0.03	2081.26 ± 26.0	2094.55 ± 21.9	7.77 ± 0.01	1282.12 ± 48.9	1.26 ± 0.03	0.75 ± 0.02
	4000		7.32 ± 0.04	2201.56 ± 41.1	2108.18 ± 30.5	7.38 ± 0.03	3278.35 ± 261.2	0.53 ± 0.03	0.32 ± 0.02
26.9 ± 0.9	ambient	17.4 ± 0.4	8.18 ± 0.10	1937.40 ± 29.0	2140.93 ± 27.4	8.28 ± 0.02	378.14 ± 22.6	4.72 ± 0.10	2.89 ± 0.07
	1400		7.83 ± 0.07	2060.81 ± 15.6	2110.25 ± 11.3	7.83 ± 0.02	1186.86 ± 65.9	1.86 ± 0.08	1.14 ± 0.05
	4000		7.44 ± 0.11	2214.92 ± 36.9	2159.79 ± 28.5	7.44 ± 0.03	3110.43 ± 249.4	0.81 ± 0.05	0.50 ± 0.04

Table 2: PERMANOVA results of the final survival as well as of the final development (proportion of nauplii metamorphosed to cyprids and post-larvae) of *Amphibalanus improvisus* nauplii in different temperature and $p\mathrm{CO}_2$ treatment combinations over 57 days using Euclidean distance matrices with 9999 permutations. Significant effects are in bold (N = 6).

		d.f.	SS	MS	Pseudo-F	p (perm)	Unique perms
Final survival	Temperature	2	0.605	0.302	20.028	**<0.001**	9959
	$p\mathrm{CO}_2$	2	0.041	0.020	1.135	0.265	9952
	Temperature × $p\mathrm{CO}_2$	4	0.147	0.037	2.429	0.064	9951
Final development	Temperature	2	0.645	0.322	21.907	**<0.001**	9946
	$p\mathrm{CO}_2$	2	0.046	0.023	1.573	0.220	9942
	Temperature × $p\mathrm{CO}_2$	4	0.166	0.041	2.818	**0.035**	9961

Table 3: Factorial ANOVA results of the larval duration (time of development from Stage-II nauplii to the cyprid) of *Amphibalanus improvisus* nauplii in different temperature and $p\mathrm{CO}_2$ treatment combinations over 57 days. Significant effects are in bold (N = 6).

	d.f.	SS	MS	F	p
Temperature	2	11.558	5.779	90.540	**<0.001**
$p\mathrm{CO}_2$	2	0.401	0.200	3.138	0.053
Temperature × $p\mathrm{CO}_2$	4	0.742	0.186	2.907	**0.032**

Table 4: Factorial ANOVA results of the final survival as well as of the final settlement of *Amphibalanus improvisus* cyprids in different temperature and $p\mathrm{CO}_2$ treatment combinations over 29 days. Significant effects are in bold (N = 6).

		SS	d.f.	MS	F	p
Final survival	Temperature	3.361	2	1.681	44.255	**<0.001**
	$p\mathrm{CO}_2$	0.283	2	0.142	3.727	**0.032**
	Temperature × $p\mathrm{CO}_2$	0.164	4	0.041	1.076	0.380
Final settlement	Temperature	0.120	2	0.060	0.990	0.380
	$p\mathrm{CO}_2$	0.123	2	0.062	1.015	0.371
	Temperature × $p\mathrm{CO}_2$	0.123	4	0.031	0.506	0.732

Figures

Figure 1: Mean salinity, temperature and pH_{NBS} for a water depth of 1 to 1.5 meter from April 2009 to May 2010 (means ± min and max over 4 different stations within the inner Kiel Fjord and the GEOMAR pier**; a**) and seasonal settlement pattern of *Amphibalanus improvisus* (means ± SE of % of year sum cover from March 2005 to October 2010; **b**) at the site of the experimental barnacle population within Kiel Fjord.

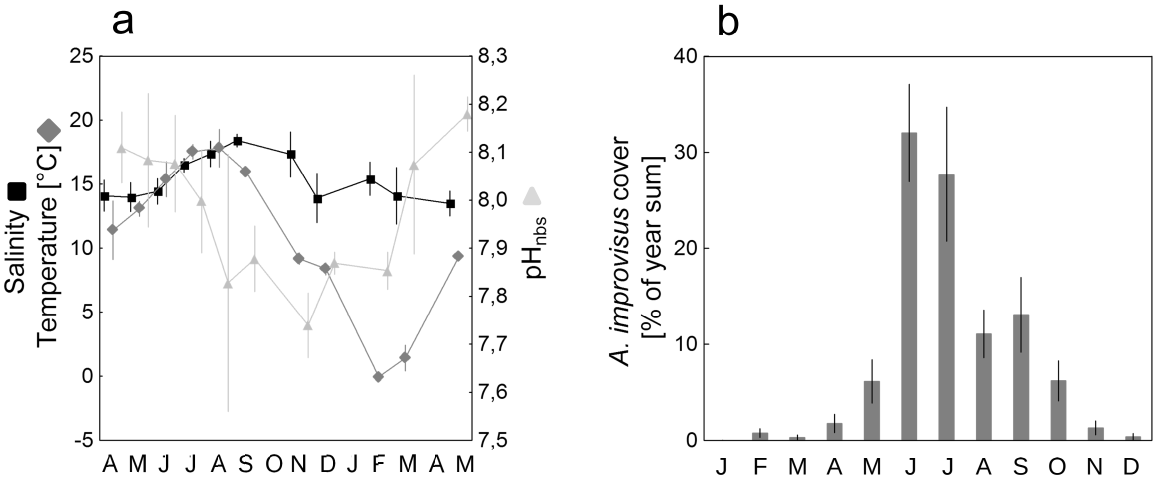


Figure 2: Scheme of the experimental setup. A single well of a 6-well plate (**a**) was filled with either filtered seawater + 20 nauplii or 10 cyprids (experimental well: dark grey wells) or filtered seawater only, left for water replacements (equilibration well: light grey wells). The 6-well plates were placed into sealed 2 l plastic aquaria (**b**) floating on 1.5 l filtered seawater, which was aerated (**c**) with pressurized air of fixed $p\mathrm{CO}_2$ concentrations. The 2 l aquaria were additionally placed in temperature baths (**d**) with different target temperatures.

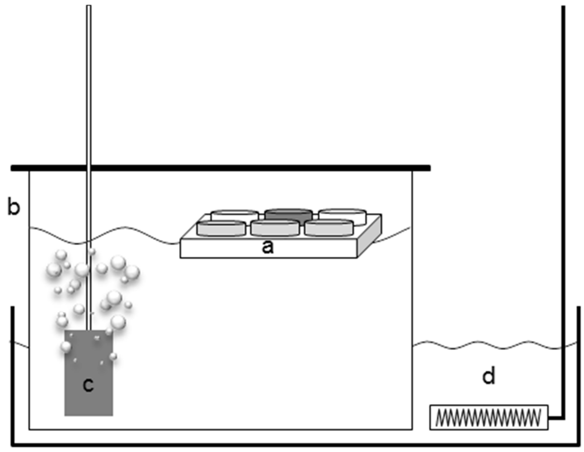


Figure 3: Survival (squares) and proportion of cyprids and post-larvae (circles) from an initial number of 20 nauplii of *Amphibalanus improvisus* in 12 °C (**a**), 20 °C (**b**) and 27 °C (**c**) at different pCO_2 treatments as well as final survival (**d**, squares) and final development (proportion of nauplii metamorphosed to cyprids and post-larvae; **d**, circles) on day 57 at different temperature × pCO_2 treatment combinations (means ± SE; N = 6). Treatment combinations differ according to PERMANOVA pair-wise tests at $p < 0.05$ when they do not share a lower case letter.

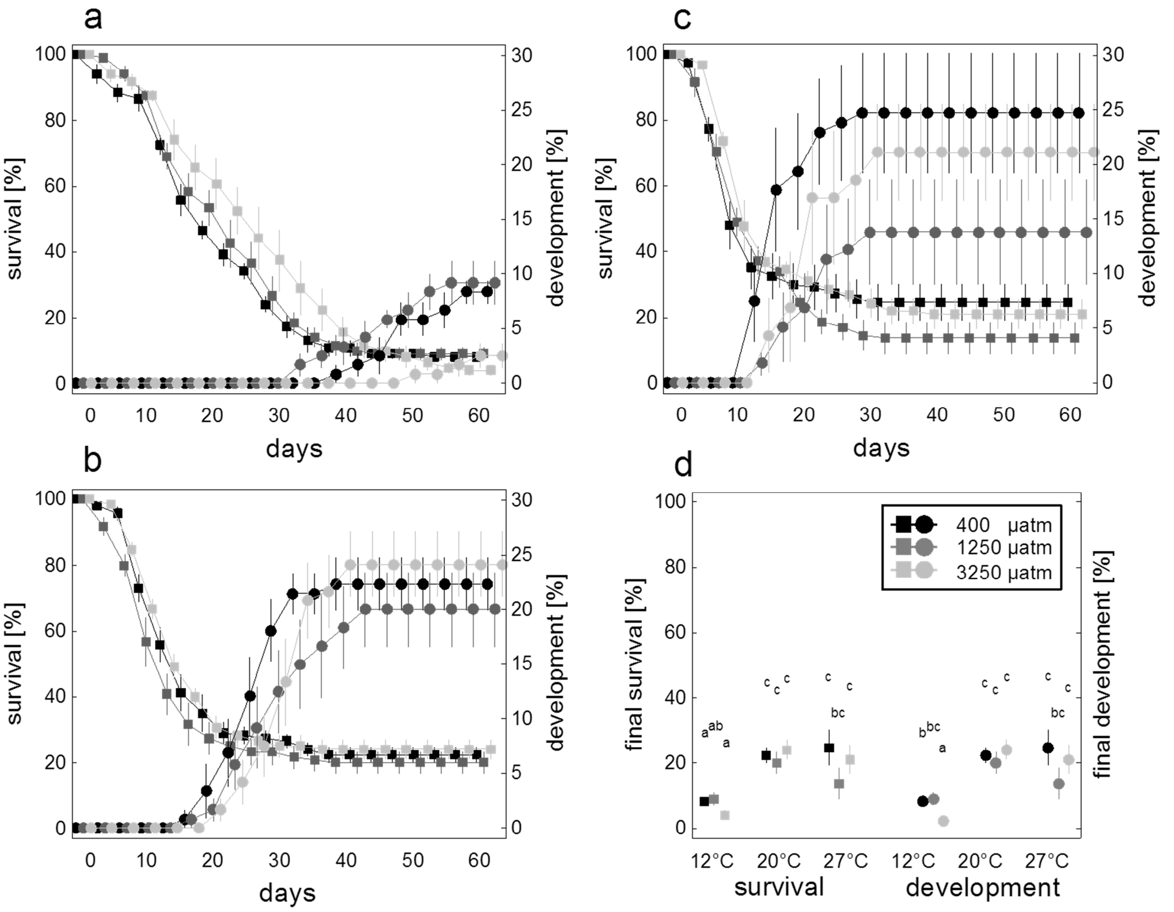


Figure 4: Larval duration (i.e. the duration from freshly hatched Stage-II nauplii to the cyprid stage) of *Amphibalanus improvisus* nauplii at different temperature × $p\mathrm{CO}_2$ treatment combinations (means ± SE; N = 6). Treatment combinations differ according to Fisher's LSD test at $p < 0.05$ when they do not share a lower case letter.

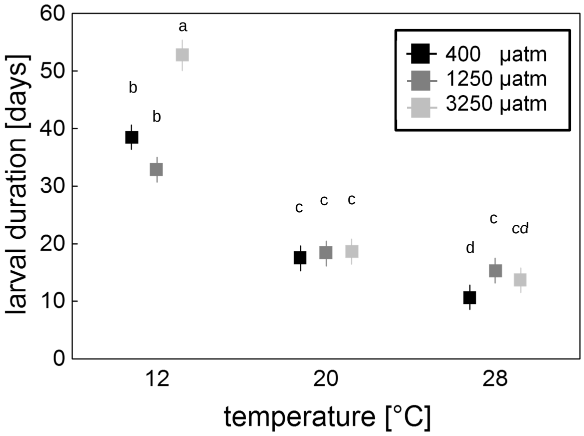
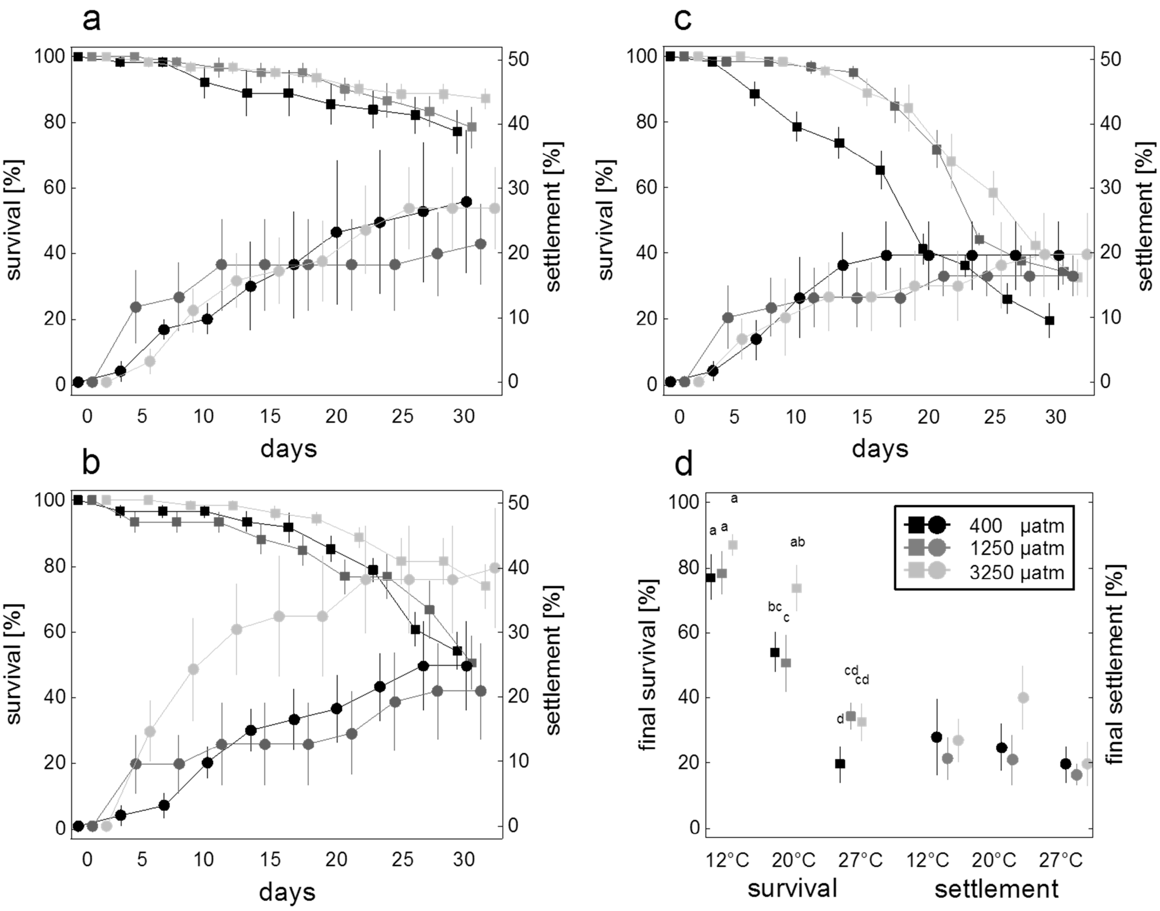


Figure 5: Survival (squares) and settlement (circles) from an initial number of 10 cyprids of *Amphibalanus improvisus* in 12 °C (**a**), 20 °C (**b**) and 27 °C (**c**) at different $p\mathrm{CO}_2$ treatments as well as final survival (**d**, squares) and final settlement (**d**, circles) on day 29 at different temperature × $p\mathrm{CO}_2$ treatment combinations (means ± SE; N = 6). Treatment combinations differ according to Fisher's LSD test at $p < 0.05$ when they do not share a lower case letter (significant differences between treatment combinations on the settlement of cyprids were absent).



Publication II – Larval development of the Tjärnö population

Larval development of the barnacle Amphibalanus improvisus in an acidified ocean.

Larval development of the barnacle *Amphibalanus improvisus* in an acidified ocean.

Christian Pansch[1*], Peter Schlegel[2,3], Martin Wahl[1], Jonathan N Havenhand[2]

[1]Department of Marine Ecology – GEOMAR | Helmholtz Centre for Ocean Research, 24105 Kiel, Germany
[2]Department of Biological and Environmental Sciences – Tjärnö, University of Gothenburg, Tjärnö, 45296 Strömstad, Sweden
[3]Department of Biological Sciences – Marine Ecology Group, Macquarie University, Sydney NSW 2109, Australia

Statement of authorship: CP, PS, MW and JNH designed the study, CP and PS collected the data, CP and PS analysed the data and wrote the manuscript and all authors contributed substantially to revisions

Corresponding author*: Tel: +49 431 600 4084
Fax: +49 431 600 1671
Email: cpansch@geomar.de

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Abstract

Increasing atmospheric CO_2 decreases seawater pH, a phenomenon known as ocean acidification (OA). Although many studies have investigated OA impacts on calcifying adult marine invertebrates, little is known about their early life-history stages. In two separate experiments we found that larval development of the barnacle *Amphibalanus improvisus* was not significantly impacted by levels of OA projected for the next 150 years. After 10 days of incubation, there were no differences in survival or overall development of larvae raised at pH 7.8 or 7.6 compared to control pH of 8.0. After 3 and 6 days of incubation, we found no significant impact of OA on development speed or larval size at pH 7.8 compared to control pH of 8.1. Our results suggest that the non-calcifying larval stages of *A. improvisus* are relatively tolerant to near future OA. This result is in line with other findings for closely related barnacle species.

Introduction

Increasing atmospheric CO_2 affects seawater pH and chemistry (Raven et al. 2005; IPCC 2007). This process, commonly known as ocean acidification (OA), has led to a decrease in oceanic seawater pH by 0.1 since the industrial revolution (Caldeira and Wickett 2005; Sabine et al. 2004). Predictions of future change depend on the emission scenario considered (Bernie et al. 2010) but models show that oceanic mean pH may fall from the current 8.1 units to 7.8 and 7.5 by 2100 and 2300, respectively (Orr et al. 2005; IPCC 2007).

OA affects marine species in various ways (Kroeker et al. 2010) and has the potential to alter fundamental structures of marine ecosystems (Hale et al. 2011). In addition to well-demonstrated impacts of OA on calcification processes (e.g. Fabry et al. 2008; Ries 2011) marine organisms may also suffer from physiological stress through impacts on their acid-base metabolism (Miles et al. 2007). This can lead negative long-term effects on metabolic functions (Pörtner et al. 2004) possibly affecting important fitness traits such as growth, survival or reproduction as demonstrated in recent meta-analyses (Kroeker et al. 2010).

Early life-history stages of marine organisms can be particularly sensitive to environmental changes (Gosselin and Qian 1997; Dupont et al. 2008; Kurihara 2008) and conditions experienced during early development may substantially affect performance in subsequent life-history stages (Thiyagarajan et al. 2002). Investigations of the effects of

OA on barnacles are, however, rare. The only studies available found that early life-history stages of the barnacle *Amphibalanus amphitrite* were not affected by OA (McDonald et al. 2009) while *A. improvisus* larvae were only affected when OA occurred in combination with temperature shifts (Pansch et al. 2012). The embryonic development of *Semibalanus balanoides* was, however, negatively impaired by decreased pH (Findlay et al. 2009). Different populations can respond differently to OA (Walther et al. 2010; Parker et al. 2011) and intra-specific (including intra-population) variation is the raw material of natural selection. This has, however, rarely been directly addressed in experimental studies. In a climate change context this omission is surprising as it gives insights of whether or not species will be able to adapt in the coming decades. Here we investigated the impacts of OA on survival and larval development of different batches of the bay barnacle *Amphibalanus improvisus*.

Materials and methods

The bay barnacle *A. improvisus* is a prominent filter-feeder in many fouling communities (Strathmann 1987; Andersson et al. 1999, 2009) and is globally widespread occurring in shallow, tidal areas in both salty and brackish waters. Eggs hatch as free-swimming stage-I nauplius larvae and develop to stage-II nauplii within 3-4 h (Thiyagarajan et al. 2003). Stage-II nauplii start feeding and go through another five moults before they develop into the non-feeding cypris stage, which settles and metamorphoses into a juvenile barnacle (post-larva; Jones and Crisp 1954).

Experiments were conducted in 2008 and 2009 at the Sven Lovén Centre for Marine Sciences in Tjärnö, Sweden. Barnacle broodstock (*A. improvisus*) comprised a mixed population of juvenile and adult individuals from Idefjorden (30 km north of Tjärnö; 59°06.2‘N, 11°21.1‘E) and the bay outside the research station (58°52.5‘N, 11°08.1‘E). Adult barnacles were kept in flow-through seawater at 20 °C and fed daily with brine shrimp (*Artemia salina*) *ad libitum*. Freshly released barnacle larvae (stage-II nauplii) were collected overnight from the broodstock tanks.

We conducted two different experiments to assess the sensitivity of *A. improvisus* larvae in response to decreased seawater pH. In the first experiment, “larval survival and development” were assessed by rearing nauplius larvae in 6-well plates over 10 days in response to 3 different pH treatments. In the second experiment “larval stage percentage and size" were assessed by rearing nauplius larvae in 5 l glass bottles over 6 days in response to 2 different pH treatments.

Larval survival and overall development

Filtered seawater (0.2 µm) was treated in 2 l glass bottles by adding pure gaseous CO_2 using computerized pH controllers (NBS scale, resolution: 0.01 units, Aqua Medic GmbH, Germany). Eight ml of filtered seawater at each of three pH levels (mean pH_{NBS} ± SD = 8.02 ± 0.06; 7.80 ± 0.04; 7.57 ± 0.08, Beckman Coulter 400 Series pH meter, NBS scale) was filled into single wells of different 6-well plates (Greiner bio-one, Smurfit Kappa GmbH, Neuburg-Germany). 20 nauplii were distributed to each of the wells. The 6-well plates were then placed in seawater-air CO_2 incubators (see Egilsdottir et al. 2009; Pansch et al. 2012 for details). These comprised sealed 11 l plastic aquaria, which were half filled with filtered seawater (0.2 µm) aerated with a mixture of fresh filtered air and pure gaseous CO_2. CO_2 concentrations were controlled using computerized pH controllers (NBS scale, resolution: 0.01 units, Aqua Medic GmbH, Germany) to provide mean pH_{NBS} (± SD) of 8.03 ± 0.04, 7.86 ± 0.02 and 7.59 ± 0.02. Larvae were reared over 10 days at 25° ± 1 °C and provided with a daily diet of marine diatoms (50:50 mixture of *Chaetoceros calcitrans* and *Skeletonema costatum*) at a concentration of 1-1.5 x 10^5 cells ml^{-1} (Strathmann 1987) under continuous light (Thiyagarajan et al. 2003). The water in the wells was exchanged every second day with filtered seawater at the respective pH.

Larval survival rates and the number of nauplii that had metamorphosed into cyprids were assessed at the end of the experiment (10 days) using a dissecting microscope (Olympus SZX12). For data evaluation, mean values from the different wells within a 6-well plate were calculated. Each treatment was replicated twice while the whole experiment was repeated 4 times (trials), using separate batches of larvae. Treatments used in each incubator were randomized between different trials.

Larval stage percentage and size

Filtered seawater (0.2 µm) was treated in 5 l glass bottles by adding pure gaseous CO_2 using computerized pH controllers. A fresh batch of nauplii was evenly distributed among the glass bottles (~ 1 larva per ml seawater) at each of two pH levels (mean pH_{NBS} ± SD = 8.09 ± 0.07; 7.80 ± 0.04). A small subsample of larvae from each bottle was fixed in 10% seawater-buffered Formalin at the beginning of the experiment. Larvae were reared over 6 days at 25 ± 1 °C. Initially, and after 3 days larvae were fed with a diet of marine diatoms (*Skeletonema costatum*) at a concentration of 1-1.5 x 10^5 cells ml^{-1} (Strathmann 1987). Larvae were held under 12:12 h day and night cycles. A full water exchange was performed after 3 days to remove detritus and faeces as well as dead larvae. Subsamples of nauplii were taken after 3 and 6 days of cultivation and fixed in 10% seawater-buffered Formalin. Each treatment was replicated three times while the whole experiment was repeated 5 times (trials) with separate batches of larvae. Incubation bottles were mixed randomly between different trials.

Nauplius stage determination (nauplius stages I-VI, cyprid = C, post-larva = P) was done according to Jones and Crisp (1954) using an inverted microscope (Olympus IX71). Larval stage percentages were determined from direct counts of each larval stage in the subsample. Total length (overall length) and carapace length of larvae were assessed according to West and Costlow (1987).

Statistical analysis

All data were tested for normality using the Shapiro-Wilk's W-test and for homogeneity of variances using Levene's test. Percentage data were arc-sine square-root transformed prior to parametric statistical analyses. When normality or homogeneity of variances was not achieved after transformation we used parametric tests but reduced the level of significance to 0.01 in order to avoid Type 1 errors (Underwood 1997, Wakefield and Murray 1998). Larval survival and overall development were tested with a factorial ANOVA with the factors trial (4 levels) and pH (3 levels). Larval stage percentage at day 3 and at day 6 were tested with a factorial ANOVA with the factors trial (5 levels) and pH (2 levels) using the percentage of larvae developed to stage V+ (day 3) or to stage C+ (day 6). Total length and carapace length were tested with a factorial ANOVA with the factors trial (5 levels) and pH (2 levels) for the dominant larval stages (total length: stage IV, V and V; carapace length: stage IV, V, VI and C). All statistical analyses were done using STATISTICA 8.0 (Stat- Soft, Inc., USA).

Results

Larval survival and overall development

Survival of larvae differed significantly between trials (i.e. different larval batches) but was not significantly affected by the pH treatment, and there was no significant interaction between trial and pH (Table 1). Survival was best in trial 3 with 79% survival of nauplii at the end of the experiment, while trial 2 showed lowest mean survival of 30% (means over the pH treatments; Fig. 1a).

Overall development of larvae after 10 days differed significantly between trials but was not significantly affected by the pH treatment, and there was no significant interaction between trial and pH (Table 1). Overall development was most rapid in trial 3 with 69% of nauplii reaching the cypris stage or metamorphosing to post-larvae by the end of the experiment (means over the pH treatments; Fig. 1b). Trial 2 showed the lowest overall development with only 15% (means over the pH treatments; Fig. 1b).

Larval stage percentage and size

The mean larval stage percentages over all 5 different trials are shown in Fig. 2. At day 3, about 68 and 25% of the larvae had developed to stage V and VI respectively (means over all trials and pHs). At day 6, about 78% of larvae had developed to the cypris stage and 3% to the post-larval stage (means over all trials and pHs). After 3 days the majority of larvae were at nauplius stage V (Fig. 2a) and after 6 days the majority were at cypris stage C (Fig. 2b). Consequently the numbers of larvae developing to these stages or later (V+ and C+ respectively) were chosen for statistical analysis of the effects of pH and trial on larval development. In both cases, there was a significant effect of trial but development rates were not significantly affected by pH, and there were no significant interactions between trial and pH (Fig. 3; Table 2).

Total length differed significantly between trials for larvae at stage V (day 3) but not for larvae at stage IV (day 3) and stage VI (day 6; Fig. 4; Table 3). Total length of larvae did not differ significantly between pH treatments and there was no significant interaction between trial and pH for any larval stage (Fig. 4; Table 3).

Carapace length differed significantly between trials for larvae at stage V (day 3) and the cypris stage (day 6) while it did not significantly differ between trials for larvae at stage IV (day 3) and stage VI (day 6; Table 4). There were no significant effects of pH nor significant interactions between trial and pH (Fig. 5; Table 4).

Discussion

Our results do not support the hypothesis that early life-history stages of *Amphibalanus improvisus* will be negatively affected by near-future levels of OA. Even relatively severe pH treatments (down to pH_{NBS} 7.6 - equivalent to scenario estimates for the year 2250; Caldeira and Wicket 2005) did not cause significant changes in survival and development rate of larvae. We also found no significant effects of OA on larval development rate or size, and most larvae reached the cypris stage after 6 days, matching development rates recorded elsewhere for this species (Semmler et al. 2009). We conclude, therefore, that early life-history stages of this species might be tolerant to near-future levels of OA.

The substantial inter-batch (trial) differences in larval development in both experiments is a common characteristic of cultures of this species (pers. obs.) and also of related species such as *Semibalanus improvisus* (Jarrett and Pechenik 1997). This likely results from heterogeneous parentage (and hence genetic composition) of larval batches, although differences may also be attributed to variability in the quality of the microalgal food supplied to the larvae (Nasrolahi et al. 2007). Interestingly, even though responses of different larval batches - and thus genetic composition - varied between trials, we found no significant interaction effects between batch and pH. This level of replication therefore strengthens our conclusion that near-future levels of OA (pH 7.8 to 7.6) are unlikely to affect larval development of *Amphibalanus improvisus*.

More generally, it has been suggested that early life-history stages of marine invertebrates are highly susceptible to environmental stress (e.g. Gosselin and Qian 1997; Kurihara 2008). Larval development of the oyster *Crassostrea gigas* and the mussel *Mytilus galloprovincialis* have been shown to be negatively impacted by pH 7.4, probably triggered by acidification impacts on calcification processes (Kurihara et al. 2007, 2009), and even small pH changes (a pH reduction by 0.2 units) have been shown to induce 100% larval mortality in the brittlestar *Ophiothrix fragilis* (Dupont et al. 2008). Similar results have been found in other calcifying species (reviewed by Ross et al. 2011; Byrne 2012), however, we did not find these patterns in our present study on the bay barnacle *A. improvisus*. This was also shown by Pansch et al. (2012) in the same species but in a distinct population. We are not alone in finding that early life-history stages of barnacles are rather tolerant to expected variations in seawater pH. This result has also been found in recent investigations of the closely related barnacle species *Amphibalanus amphitrite* (McDonald et al. 2009). As barnacle larvae do not develop calcified structures until they settle and metamorphose into post-larvae, they may be less susceptible to OA stress compared to other invertebrate larvae (Kurihara et al. 2007; Dupont et al. 2008; Kurihara 2008, Kurihara et al. 2009). Crustaceans generally are known to have high metabolic rates that facilitate control of extracellular pH through active ion transport (Gohad et al. 2009;

Whiteley 2011). Consequently even at the larval stage, they may be capable of tolerating variations in seawater pH (e.g. Kurihara et al. 2004; Arnold et al. 2009).

Although survival, size, and development rates of *Amphibalanus improvisus* larvae were not affected by pH, other processes, which are vital for later settlement and recruitment success of juveniles may have been impacted (Thiyagarajan et al. 2002). Barnacle nauplii generate lipid vesicles, which are used as energy reservoirs during the non-feeding cyprid stage, for swimming, settlement and metamorphosis. These reservoirs have a profound importance for growth of barnacle post-larvae (Thiyagarajan et al. 2002, 2003). Hypercapnia induced stress may reduce the ability of larvae to build up sufficient lipid vesicles, leading to fatal effects for subsequent life-history stages, e.g. reduced settlement success and decreased juvenile recruitment (Beckerman et al. 2002). These issues have yet to be addressed in this species.

Detailing the effects of elevated $p\mathrm{CO_2}$ levels on other life stages of barnacles becomes difficult, as there are few data available. Nothing is known about OA impacts on the fertilization process yet, but slower embryo development was found in *Semibalanus balanoides* at pH 7.7 (Findlay et al. 2009), which could lead to a delayed release of larvae into the water. The authors argue that, as larval release is synchronized with the phytoplankton spring bloom, a delay here could decrease larval survival rates as a result of insufficient food supplies. In our experiments it was not possible to detail this effect as the used larvae developed and hatched in ambient seawater and were afterwards provided with sufficient food during cultivation.

Juvenile and adult barnacles may be robust to near-future OA, and may even withstand extreme pH minima (pH 6.6) for a short time by closing their rostral plates completely (Hall-Spencer et al. 2008). However, when faced with long-term acidification (pH 7.7) the barnacle *Elminius modestus* exhibited reduced growth, with no impacts on shell calcium content and survival, and *Semibalanus balanoides* showed reduced shell calcium content and survival under the same OA in combination with future warming (+5 °C; Findlay et al. 2009, 2010).

A range of biological and abiotic processes can cause the pH of shallow coastal habitats to vary substantially over the year or even on a daily basis (Blackford and Gilbert 2007; Shim et al. 2007; Salisbury et al. 2008; Wootton et al. 2008; Feely et al. 2010; Thomsen et al. 2010). Species inhabiting these habitats such as the barnacle *A. improvisus* will likely have undergone selection for tolerance to these fluctuating conditions, and may therefore be better able to cope with climate change than organisms from more stable habitats (Miller et al. 2009; Parker et al. 2011; Pansch et al. 2012). Whether, and how, entire barnacle populations will cope with near-future levels of ocean acidification remains unclear as it is difficult to draw conclusions from the few available studies.

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Tables

Table 1: Factorial ANOVA on the effects of pH and trial (larval batch) on larval survival and overall development. Significant effects are underlined.

		SS	d.f.	MS	F	p
survival	trial	1.013	3	0.338	24.663	<0.001
	pH	0.023	2	0.011	0.822	0.463
	trial × pH	0.071	6	0.012	0.861	0.549
overall development	trial	0.674	3	0.225	23.764	<0.001
	pH	0.028	2	0.014	1.471	0.268
	trial × pH	0.038	6	0.006	0.674	0.673

Table 2: Factorial ANOVA on the effects of pH and trial (larval batch) on the percentage of larvae developed to stage V and further (stage V + VI + C + P = V+) on day 3 and to stage C and further (stage C + P = C+) at the end of the experiment on day 6. Significant effects are underlined.

		SS	d.f.	MS	F	p
stage V+ (day 3)	trial	0.645	4	0.161	79.694	<0.001
	pH	0.006	1	0.006	3.116	0.093
	trial × pH	0.008	4	0.002	0.935	0.464
stage C+ (day 6)	trial	0.936	4	0.234	240.109	<0.001
	pH	0.001	1	0.001	0.639	0.434
	trial × pH	0.004	4	0.001	1.079	0.393

Table 3: Factorial ANOVA on the effects of pH and trial (larval batch) on the total length of nauplius larvae (stage IV, V and VI). Significant effects are underlined.

		SS	d.f.	MS	F	p
stage IV	trial	2144.2	4	536.0	3.042	0.041
	pH	1337.3	1	1337.3	7.590	0.012
	trial × pH	2931.0	4	732.8	4.159	0.013
stage V	trial	4158.6	4	1039.6	14.417	<0.001
	pH	97.7	1	97.7	1.355	0.258
	trial × pH	209.9	4	52.5	0.728	0.583
stage VI	trial	625.4	4	156.4	0.433	0.783
	pH	273.1	1	273.1	0.756	0.395
	trial × pH	2229.7	4	557.4	1.543	0.228

Table 4: Factorial ANOVA on the effects of pH and trial (larval batch) on the carapace length of nauplius (stage IV, V and VI) and cypris larvae (stage C). Significant effects are underlined.

		SS	d.f.	MS	F	p
stage IV	trial	1835.1	4	458.8	2.499	0.079
	pH	567.4	1	567.4	3.091	0.096
	trial × pH	930.6	4	232.7	1.268	0.319
stage V	trial	10239.6	4	2559.9	9.921	<0.001
	pH	1625.5	1	1625.5	6.300	0.021
	trial × pH	2884.3	4	721.1	2.795	0.054
stage VI	trial	1077.5	4	269.4	2.214	0.104
	pH	0.8	1	0.8	0.007	0.936
	trial × pH	989.9	4	247.5	2.034	0.128
stage C	trial	3843.3	4	960.8	29.065	<0.001
	pH	55.7	1	55.7	1.684	0.210
	trial × pH	98.6	4	24.7	0.746	0.573

Figures

Figure 1: Percent survival (**a**) and percent development to cyprids or post-larvae (**b**) after 10 days in pH 8.0 (black bars), pH 7.8 (dark grey bars) and pH 7.6 (light grey bars) for 4 different trials (mean ± 95% CI).

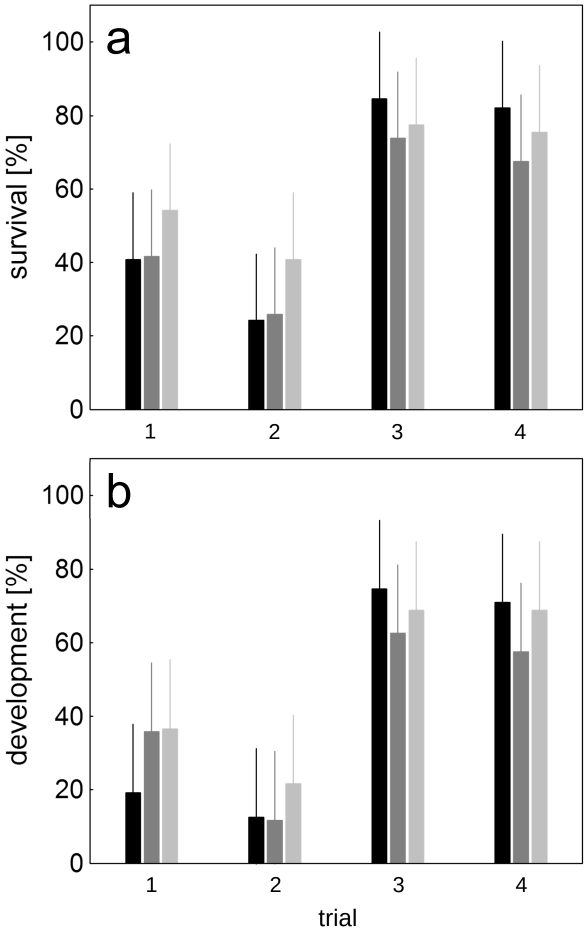


Figure 2: Percentage of larvae in different developmental stages (nauplius larvae = II-VI, cypris = C and post-larvae = P) on day 3 (**a**) and day 6 (**b**) in pH 8.1 (black bars) and pH 7.8 (dark grey bars; means over all 5 trials ± 95% CI).

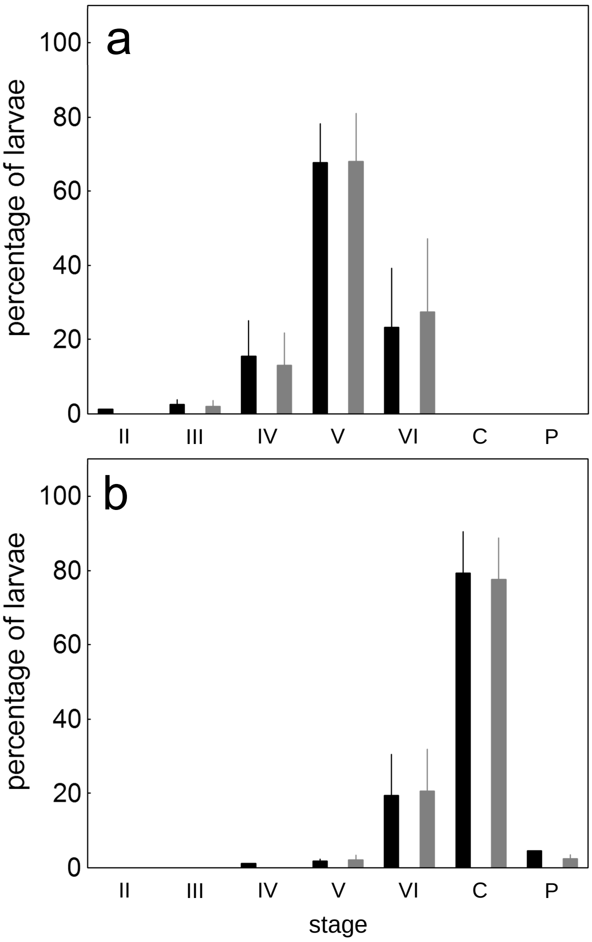


Figure 3: Percentage of larvae developed to stage V and further on day 3 (**a**) and to stage C and further on day 6 (**b**) in pH 8.1 (black bars) and pH 7.8 (dark grey bars) over 5 different trials (means ± 95% CI).

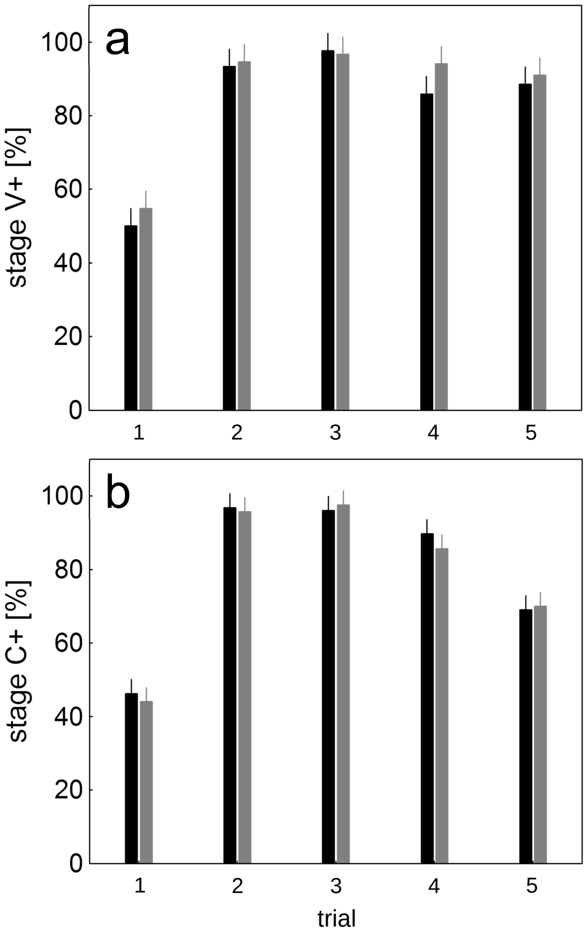


Figure 4: Total length of nauplius larvae at stage IV (**a**) and stage V (**b**) on day 3 and stage VI (**c**) on day 6 in pH 8.1 (black bars) and pH 7.8 (dark grey bars) over 5 different trials (means ± 95% CI).

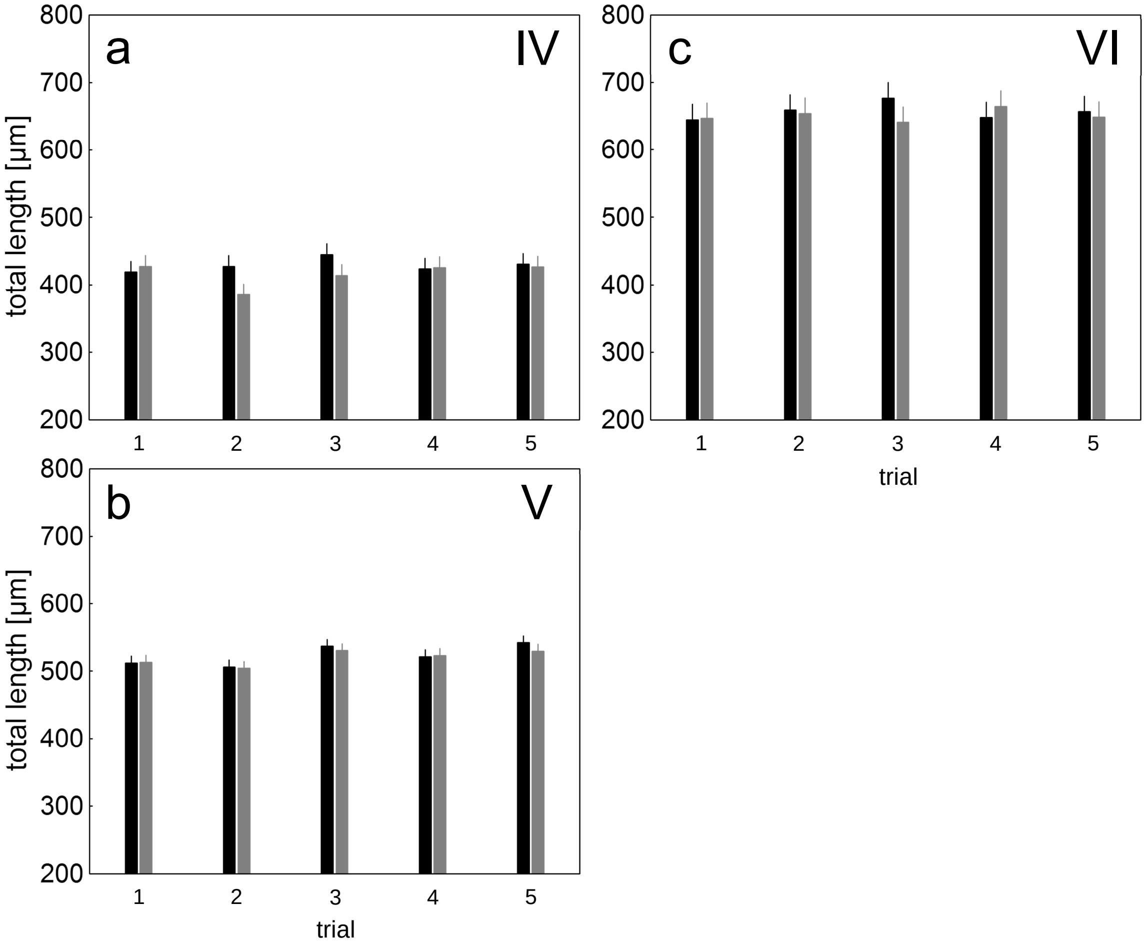
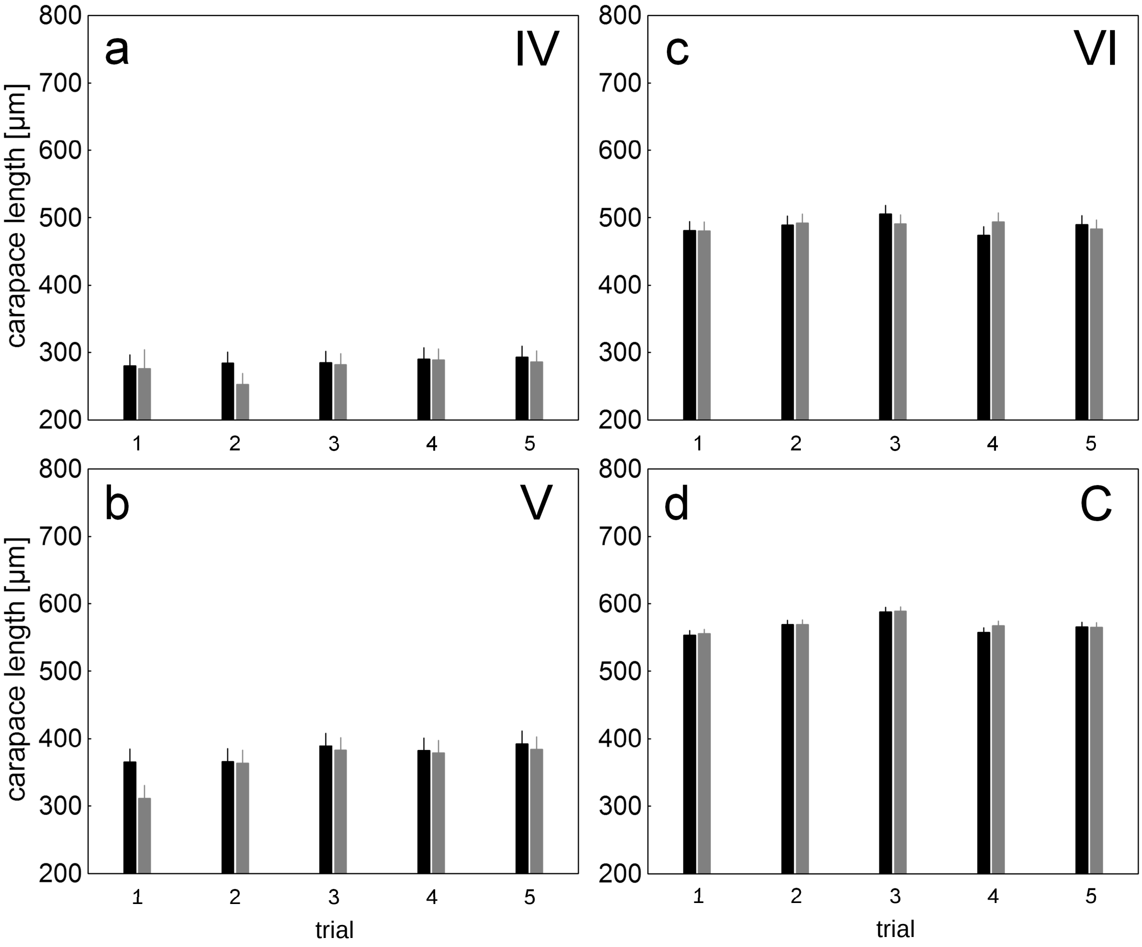


Figure 5: Carapace length of nauplius larvae at stage IV (**a**) and stage V (**b**) on day 3 and of stage VI (**c**) and cyprids (**d**) on day 6 in pH 8.1 (black bars) and pH 7.8 (dark grey bars) over 5 different trials (means ± 95% CI).



Publication III – Juvenile development of the Kiel population

Ocean warming and ocean acidification - impacts on juvenile Amphibalanus improvisus.

Ocean warming and ocean acidification - impacts on juvenile *Amphibalanus improvisus*.

Christian Pansch*, Ali Nasrolahi, Yasmin Appelhans, Martin Wahl

Statement of authorship: CP and MW designed the study, CP and AN collected the data, CP analysed the data and wrote the manuscript and all authors contributed substantially to revisions

Corresponding author*:

Tel: +49 431 600 4084
Fax: +49 431 600 1671
Email: cpansch@geomar.de

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Abstract

We investigated warming and ocean acidification (OA) impacts on newly settled *Amphibalanus improvisus* from Kiel Fjord, an estuarine ecosystem characterized by significant natural pCO$_2$-variability. In two subsequent experiments, juvenile barnacles were maintained at two temperature and three OA scenarios (20/24 °C, 700-2140 µatm, batch culture, 8 weeks) and four OA scenarios (20 °C, 620-2870 µatm, flow-through system, 12 weeks). Warming enhanced growth and shell-strength of barnacles and led to more investment into shell- compared to body growth. OA (2140 µatm) enhanced growth initially (week 2), did not affect the condition index but reduced shell-strength of barnacles. In the second experiment, barnacle growth was enhanced under moderate OA (1030 µatm) but compromised under severe OA (1930, 2870 µatm). Condition index and shell-strength were not impacted by any OA level. These results demonstrate a strong tolerance of *A. improvisus* towards predicted near future OA with negative long-term impacts in extremely acidified waters.

Introduction

Anthropogenic CO$_2$ emissions have led to an increase in CO$_2$ uptake by the oceans (Sabine et al. 2004; IPCC 2007). Dissolved CO$_2$ decreases the pH of seawater, a phenomenon called “ocean acidification” (OA; Feely et al. 2004; Orr et al. 2005). Ocean models predict an increase in seawater pCO$_2$, possibly exceeding concentrations of 1000 µatm by the year 2100 and 2000 µatm by the year 2300 (decrease in 0.4 and 0.8 pH units, respectively; Caldeira and Wickett 2005; Orr et al. 2005; IPCC 2007). These changes in water chemistry will ultimately lower saturation states (Ω) of calcite, aragonite and Mg-calcite (Orr et al. 2005; Andersson et al. 2008); important parameters for calcification processes in marine organisms. Thus, OA could affect central biological processes and indirectly alter fundamental structures of pelagic and benthic marine ecosystems (Harley et al. 2006; Riebesell 2008, Hale et al. 2011).

Recent reviews have summarized the previous findings on a wide range of impacts of OA on marine organisms (Fabry et al. 2008; Kurihara 2008; Doney et al. 2009; Dupont et al. 2010, Gattuso and Hansson 2011). A study on OA effects in 18 benthic marine organisms from diverse taxonomic groups showed significantly reduced calcification in 10 of the species, while 7 species exhibited increased net-calcification (Ries et al. 2009). First comprehensive meta-analyses revealed predominantly negative but highly variable responses of invertebrates (Hendriks et al. 2010; Kroeker et al. 2010). Calcifying organisms typically exhibit larger negative responses than non-calcifiers. Crustaceans, however, showed to be exceptionally tolerant to OA in these meta-analyses, possibly explained by their ability of controlling extracellular pH through active ion transport (Melzner et al. 2009; Whiteley 2011).

In nature, ocean acidification is not an isolated stressor but will always act together with other predicted environmental shifts such as ocean warming (Meier 2006; IPCC 2007; The BACC author team 2008). When acting simultaneously with OA or other abiotic stressors, warming effects for marine ectotherms might be even stronger due to a stress-driven narrowing of the thermal tolerance window (Pörtner 2008; Pörtner and Farrell 2008; Pörtner 2010; Walther et al. 2010). Warming also has the potential to mitigate negative effects of OA as was recently shown for sea urchin larvae and oysters (Brennand et al. 2010; Waldbusser et al. 2011).

As reported from other coastal areas (Blackford and Gilbert 2007; Shim et al. 2007; Feely et al. 2008; Salisbury et al. 2008; Wootton et al. 2008; Miller et al. 2009; Feely et al. 2010) the Kiel Fjord (Western Baltic Sea) is characterized by strong fluctuations in water $p\mathrm{CO_2}$ and pH mainly due to photosynthesis, heterotrophic degradation and river discharges (Rabalais et al. 2002; Diaz and Rosenberg 2008; Salisbury et al. 2008) with particularly strong temporary acidified conditions in even shallow habitats (Thomsen et al. 2010). These particularities of coastal waters further amplify acidification driven by atmospheric $\mathrm{CO_2}$ uptake (Melzner et al. 2012). Investigations in the vicinity of volcanic $\mathrm{CO_2}$ vents in the Mediterranean show a close correlation of the degree of $\mathrm{CO_2}$ mediated acidification and the occurrence of calcifying organisms (Hall-Spencer et al. 2008; see also Fabricius et al. 2011). In such small and gradually acidified areas, adaptation to increased $\mathrm{CO_2}$ may be hindered by gene flow from adjacent non-impacted areas. Adaptation, however, seems possible when entire regions such as the Baltic Sea with restricted genetic exchange with the oceans, feature temporarily acidified conditions. Due to possible adaptation as well as barnacles' high plasticity in tolerating strong fluctuations in environmental conditions (Wong et al. 2011), local barnacle populations might exhibit reduced sensitivity to future OA.

An earlier study on barnacles showed a decrease in *Elminius modestus* growth under OA (1000 µatm $p\mathrm{CO_2}$, pH ~7.7) with no impacts on shell calcium content and survival, while *Semibalanus balanoides* exhibited no reduction in growth but showed a reduction in shell calcium content and survival under future warming (+5 °C) and OA

(Findlay et al. 2009; Findlay et al. 2010a). A modelling approach on a *Semibalanus balanoides* population showed the combined effects of warming and OA to have stronger negative implications than warming alone (Findlay et al. 2010c). Larval condition, cyprid size, cyprid attachment and metamorphosis, juvenile to adult growth, egg production, net calcification or shell-strength of the basal region of the shell plate of the barnacle *Amphibalanus amphitrite* were not or rather positively affected by a decrease in 0.6 pH units (McDonald et al. 2009). Thus, the sensitivity of barnacles to OA apparently varies between taxonomic groups, life stages, habitats or the degree of OA stress applied.

Barnacles are an important component of estuarine ecosystems within the species poor Baltic Sea (Berntsson and Jonsson 2003) and growth as well as shell-strength are critical ecological traits determining population dynamics (Urban 2007). Rapid juvenile growth after successful settlement and metamorphosis is a particularly important determinant of recruitment success (Thiyagarajan et al. 2007). Environmental shifts affecting barnacle growth such as warming and food availability (Sanford and Menge 2001; Skinner et al. 2007) or OA (Findlay et al. 2009; Findlay et al. 2010a) will likely affect recruitment and population dynamics of barnacles.

While larval stages of the barnacle *Amphibalanus improvisus* were shown to be rather tolerant to OA (Pansch et al. 2012), to our knowledge, no studies have investigated the combined effects of warming and OA upon juveniles of *A. improvisus*. In the past, most studies focused on single rather than multiple stress effects in short term rather than long term experiments. In this study we investigate the sensitivity of *A. improvisus* juveniles to realistic combinations of future warming and OA in long-term experiments.

Materials and methods

On a cruise in September 2009, water samples from various shallow benthic habitats along the Western Baltic coast were taken by scuba diving. In-situ salinity and temperature were measured using a WTW Cond 340i equipped with a TetraCon® 325 electrode. C_T (dissolved inorganic carbon) was measured coulometrically (SOMMA System autoanalyser). A_T (total alkalinity) was determined by potentiometric titration (VINDTA autoanalyser). Measurements were corrected using DICKSON seawater standard as reference (Dickson et al. 2003). All other parameters (pH_T - total scale, pCO_2, $\Omega_{Aragonite}$ and $\Omega_{Calcite}$) were calculated using CO_2 SYS macro (Pierrot et al. 2006), with dissociation constants (K_1 and K_2) according to Mehrbach et al. (1973) and refitted by Dickson and Millero (1987) using $KHSO_4$ dissociation constant after Dickson (1990).

All lab experiments were conducted in constant temperature rooms of the GEOMAR, Kiel, Germany in late summer and autumn 2010. Juveniles of the barnacle *Amphibalanus improvisus* were collected by exposing transparent settlement panels (Perspex®, 9 × 9 cm; horizontally) to the subtidal zone of the inner Kiel Fjord (54°19.5‘N, 10°09.0‘E) for two weeks; with barnacles being able to only settle on the bottom side. Surplus barnacles were gently removed and the panels were distributed to the different treatment combinations. We conducted two subsequent experiments, using a batch culture in which two temperature (20 and 24 °C) and three pCO_2 (mean pCO_2: 700, 1000 and 2140 µatm) treatments were applied as well as a flow-through water system with four different pCO_2 treatments (mean pCO_2: 620, 1030, 1930 and 2870 µatm). Temperature treatments were chosen following mean late summer temperatures within Kiel Fjord (~20 °C) and projected warming of surface water within the Baltic Sea (+ 4 °C). The noticeably high pCO_2 levels were chosen following the high natural variability in pCO_2 within the habitat of the investigated barnacle population taking predicted future scenarios into account (Thomsen et al. 2010; Wahl et al. 2010; Melzner et al. 2012).

Temperature and pH_{NBS} (NBS-scale) measurements over the experiments were done weekly using a WTW 330i pH meter equipped with a SenTix® 81 pH electrode. Salinity was measured using a WTW Cond 340i equipped with a TetraCon® 325 electrode. Additional water samples for C_T and pH_T measurements were taken every second week. C_T was measured coulometrically (AIRICA C_T analyser; Marianda, Kiel, Germany) and pH_T was measured using an 826-Metrohm mobile pH meter equipped with a Methrom 6.0262.100 electrode. Measurements were corrected using DICKSON seawater standard as reference material (Dickson et al. 2003). All other parameters (pCO_2, A_T, $\Omega_{Aragonite}$ and $\Omega_{Calcite}$) were calculated as mentioned above.

Barnacle growth was assessed by measuring basal-plate diameter and total dry weight (DW) of the barnacles (i.e. including body and shell mass). To determine the maximum basal-plate diameter, images from the backside of each transparent settlement panels were taken with a digital camera (CANON EOS 20D, EFS 18-55mm) and evaluated using image analysis software (ImageJ 1.43u). The measures of each single barnacle on a settlement panel provided a mean barnacle size for each settlement panel representing one replicate. Natural barnacle growth in the field was assessed by exposing settlement panels to the Kiel Fjord in 1 meter depth at the starting day of the respective lab experiment with biweekly measurements of the basal-plate diameter as described for the lab experiments. Newly settled barnacles on the panels in the field were regularly removed to allow unhindered growth of the measured individuals. Temperature loggers (HOBO®, Onset Computer) were used to monitor the water temperature at the site of the respective field controls.

To observe the growth of barnacles using DW measurements, 3-5 barnacles were randomly scraped from the panels at each sampling day and frozen at -20 °C. At the end of each experiment, barnacles were dried at 80 °C (MMM Ecocell) for 3 days and weighed (Sartorius, ± 0.1 mg). The dried samples were additionally burned for 20 h at 500 °C (Nabertherm B150) and the remaining inorganic material was weighed (ash weight, AW). We calculated ash-free dry weight (AFDW) by subtracting AW from DW. The condition index (CI; body to shell ratio) for juvenile barnacles was calculated by dividing the AFDW by the AW. Mean DW and mean CI were calculated for each settlement-panel.

The force required to dislodge the barnacles from the settlement panel (shear force) as well as the force required to break the barnacle shell (breaking force) was determined with a TAXT2i texture analyser (Stable Micro Systems, 25-1 measuring cell) with a speed of 1.0 mm s^{-1}. Maximum shear force was measured with a 1 cm wide rabble scraping the barnacle off the panel surface (parallel to the substrate; Fig. 1). Maximum breaking force was measured using a cylinder of 2 mm diameter pushing onto the rostrum/rostrumlateral-plate (Fig. 1). Shear and breaking forces were corrected for size (basal diameter) for each measured individual and will be given as g per mm^{-1}. For further analysis, mean shear- and breaking force were calculated for each single settlement panel.

Batch culture experiment

The first experiment was run in 1 l plastic beakers filled with filtered seawater (0.3 µm; mean salinity ± s.d.: 15.3 ± 1.1) placed in temperature controlled (Aqua Medic®, Germany) water baths with target temperatures of 20 and 24 °C (mean temperatures ± s.d.: 20.0 ± 0.3; 24.0 ± 0.5). The different pCO_2 treatments were achieved by directly bubbling ambient air or pre-mixed gas with pCO_2 concentrations of 1120 or 4000 ppm into the beakers. Mean pCO_2 values across the different temperatures (i.e. 700, 1000 and 2140 µatm pCO_2), in the following, will indicate the acidification treatment levels. Each treatment was replicated 8 times.

Juveniles (mean size ± s.d.: 0.98 ± 0.07 mm) were “captured” on settlement panels (exposed in-situ for 2 weeks) and reared in the lab for 2 months using a 12/12 h day/night cycle (~25 µmol photons $m^{-2}s^{-1}$). Each experimental unit consisted of two separate 1 l plastic beakers. While the panels were placed within one of the beakers, the water in the remaining beaker could independently adjust to the respective temperature and $p\mathrm{CO}_2$ values for two days. The panels were alternated between these beakers every other day with the water in the beaker without a panel being replaced immediately after the transplantation. Barnacles were fed with marine diatoms (1:1 mixture of *Chaetoceros calcitrans* and *Skeletonema costatum*; 2×10^5 cells per ml) every other day. Starting on day 24, barnacles were additionally fed with brine shrimps (*Artemia salina*; ~7.5 individuals per ml seawater). To keep the biomass of barnacles per beaker approximately constant, the amount of 50 barnacles per beaker at the beginning of the experiment was reduced to 40 on day 14, to 30 on day 35, to 24 on day 36 and to 20 on day 42. Images of the barnacles were taken every second week and samples for DW and CI analysis were taken on day 14, 35, 42 and day 56. Resistance to dislodgement and breakage were tested at the end of the experiment (day 62).

Flow-through experiment

The second experiment was run in 3.5 l plastic aquaria (covered with a plastic lid) connected to a flow-through water system. Unfiltered seawater (sand filter), pumped directly from the Kiel Fjord into the temperature rooms, was adjusted to a constant water temperature of 20 °C and pumped into a header tank from which each single aquarium was supplied with a constant water flow of ~1.6 l h^{-1} (mean temperature in the aquaria ± s.d.: 19.6 ± 0.6). The different $p\mathrm{CO}_2$ treatments were achieved by directly bubbling ambient air or pre-mixed gas with $p\mathrm{CO}_2$ concentrations of 1120, 2400 or 4000 ppm into the aquaria. Mean $p\mathrm{CO}_2$ values (i.e. 620, 1030, 1930, 2870 µatm $p\mathrm{CO}_2$), in the following, will indicate the acidification treatment levels. Each treatment was replicated 5 times.

Juveniles (mean size ± s.d.: 1.39 ± 0.06 mm) were “captured” on settlement panels (exposed in-situ for 2 weeks) and reared for 3 month using a 12/12 h day/night cycle (~6-10 µmol photons $m^{-2}s^{-1}$). Barnacles were fed daily with marine diatoms and with brine shrimps (from day 36) in concentrations as mentioned above. The amount of 17 barnacles per aquarium at the beginning of the experiment was reduced to 14 on day 28 and to 11 on day 56. The diameter of the barnacles was measured every second week and samples for

DW and CI analysis were taken on day 28, 56 and 85. The resistance to dislodgement and breakage were tested at the end of the experiment (day 86).

Statistical analysis

For experiment-I, a fully crossed two-factorial design was used with temperature (2 levels) and $p\mathrm{CO}_2$ (3 levels) as the fixed factors. For experiment-II, $p\mathrm{CO}_2$ (4 levels) was the fixed factor. We tested for normality with the Shapiro-Wilk's W-test. Non-normal data were transformed to normality prior to the use of parametric statistics. Percentage data were arcsine transformed. We tested for homogeneous variances using Levene's test. Data from growth measurements (diameter and DW) and CI were evaluated using repeated measures ANOVA, the fixed factors being temperature and $p\mathrm{CO}_2$ for experiment-I and $p\mathrm{CO}_2$ for experiment-II with time (days) as the RM factor. When the data did not meet the assumption of sphericity (Mauchly‘s test for sphericity), p-values were corrected using the Greenhouse-Geisser correction. To analyse the shear force as well as the breaking force data, we used a two-factorial ANOVA ($p\mathrm{CO}_2$ × temperature) for experiment-I and a one-way ANOVA for experiment-II. We used Fisher's least significant difference (LSD) tests for multiple comparisons following ANOVA. The relationship of AFDW and AW was evaluated using simple regression analysis. All statistics were performed using the software STATISTICA 8.0 (Stat- Soft, Inc., USA).

Results

The carbonate system measured in various shallow benthic habitats within the western Baltic Sea showed $p\mathrm{CO}_2$ concentrations ranging between 329 and 1424 µatm and $\mathrm{pH}_{\mathrm{NBS}}$ values between 8.13 and 7.55 (Table 1). Overall mean $p\mathrm{CO}_2$ and $\mathrm{pH}_{\mathrm{NBS}}$ over all habitats and depths were 698 µatm and 7.86, respectively. Even very shallow waters exhibited undersaturation with respect to aragonite as seen for Schleimünde (2m), Staberhuk (3m) or Salzhaff (3.3m). Undersaturation with respect to calcite was only found in Schleimünde at 15m depths.

The carbonate system of both lab experiments is represented in Table 2. Seawater was undersaturated with respect to aragonite in all treatments with artificially elevated $p\mathrm{CO}_2$ but also in 20 °C and control $p\mathrm{CO}_2$ of the batch culture experiment. Seawater was undersaturated with respect to calcite in treatments with $p\mathrm{CO}_2$ levels of 1930 µatm and higher. The incoming Fjord water was undersaturated with respect to aragonite but not calcite during the water flow-through experiment with mean $p\text{CO}_2$ values of 1001 µatm (Table 2). These particularities of the incoming Fjord water explain the high $p\text{CO}_2$ values of 700 and 620 µatm during the lab experiments considered to as control conditions. The carbonate system of the batch culture experiment showed a much higher variability in measured pH and C_T as well as in calculated A_T than the water flow-through experiment. This is most likely due to the strong calcification processes by barnacles reducing the alkalinity as well as the pH and water exchanges being conducted every two days only.

Barnacle growth in the field is presented in Fig. 2. Mean growth rates were 0.12 mm × d^{-1} for cohort-I and 0.10 mm × d^{-1} for cohort-II reaching a mean size 7.82 mm and 7.16 mm in diameter after 56 days, respectively. From December 2010, growth rates of juvenile barnacles (cohort-II) were reduced over the winter months but increased in April and May 2011 with rates of 0.06 mm × d^{-1}. Water temperatures in 1 meter depth at the natural barnacle site reached maximum values of up to 23.4 °C during July but decreased gradually from August to December to minimum values down to -0.6 °C in late December and early January. From March 2011, water temperatures increased gradually (Fig. 2).

Batch culture experiment

Repeated measures ANOVA of barnacle size measurements in the batch culture experiment showed a significant effect of temperature and an interaction between day and $p\text{CO}_2$ (Table 3). Barnacles grew faster at 24 °C than at 20 °C (particularly so in the beginning of the experiment), resulting in an 8% larger diameter on day 14 (mean over the $p\text{CO}_2$ treatments; Fig. 3a). Increased $p\text{CO}_2$ (2140 µatm) led to a 9% increased basal diameter on day 14 compared to 700 µatm (mean over the both temperatures; Fig. 3a). Temperature as well as $p\text{CO}_2$ effects, however, disappeared towards the end of the experiment (Fig. 3a). Barnacles showed mean growth rates of 0.13 mm × d^{-1} reaching 8.22 mm in diameter after 56 days (20 °C, 700 µatm $p\text{CO}_2$; Fig. 3a).

Warming initially enhanced the increase in DW but this effect disappeared towards the end of the experiment (Fig. 3b; Table 3). All other factors and interactions were not statistically significant (Table 3). Warming led to an overall decrease of the CI of barnacles by 17% (mean over all days and $p\text{CO}_2$ treatments; Fig. 3c; Table 3). Highest condition indices were observed on day 35 (Fig. 3c).

The shear force required to dislodge the barnacles from the settlement panels was significantly enhanced by warming, independently of the $p\text{CO}_2$ treatment applied (Fig. 3d;

Table 3). Warming enhanced the resistance to breakage while high levels of pCO_2 (2140 µatm) significantly decreased the breaking resistance of barnacles (Fig. 3d; Table 3).

Flow-through experiment

Repeated measures ANOVA of barnacle size measurements of the water flow-through experiment showed a significant effect of pCO_2 and an interaction between day and pCO_2 (Table 4). Growth of barnacles was favoured under moderate and compromised under enhanced OA (Fig. 4a). On day 84, barnacle mean diameter was decreased by approximately 9 and 5% under pCO_2 values of 1930 and 2870 µatm, respectively. Mean size under 1030 µatm was, however, increased by 5% compared to the control treatment (Fig. 4a). Barnacles showed mean growth rates of 0.11 mm × d^{-1} reaching 7.59 mm in diameter after 56 days (20 °C, 620 µatm pCO_2; Fig. 4a).

In comparison to the controls, DW was significantly lower under 1930 and 2870 µatm pCO_2 on day 85, and significantly higher under 1030 µatm (Fig. 4b; Table 4). The CI of *A. improvisus* was not affected by pCO_2 (Table 4). Highest condition indices were observed on day 56 (Fig. 4c).

Neither the shear force required to dislodge the barnacles from the settlement panels nor the resistance to breakage were significantly affected by the pCO_2 treatments applied (Fig. 4d; Table 4).

Discussion

In-situ measurements within various shallow habitats along the western Baltic coast revealed these waters to be highly variable habitats with respect to their carbonate chemistry. Nevertheless, even areas temporarily undersaturated with respect to calcite or aragonite are dominated by calcifying species such as *Mytilus edulis* or *Amphibalanus improvisus*. This has also been observed for the temporarily acidified inner Kiel Fjord (Thomsen et al. 2010) where both calcifying species are abundant, dominating most hard substrata accessible (Dürr and Wahl 2004).

Barnacles within the inner Kiel Fjord showed high growth rates (0.10-0.12 mm basal diameter per day) and, thus, most likely reached maturity within two months (*Amphibalanus amphitrite* at growth rates of 0.10 mm per day: McDonald et al. 2009). Growth paused during the winter months, when food availability and water temperatures were low. Earlier investigations described Kiel Fjord as an estuarine ecosystem characterized by significant natural pH (pCO$_2$) -variability with highest acidification occurring during autumn (Thomsen et al. 2010). Nevertheless, we observed noticeably high growth rates and, thus, rates of calcification, even in times of naturally acidified water conditions.

Corroborating these findings in the field, both lab experiments show a remarkable tolerance of juvenile *A. improvisus* to OA levels predicted for the open ocean by the end of this century (Caldeira and Wickett 2005; Orr et al. 2005) but already temporarily encountered by barnacles within their natural habitats already today (Thomsen et al. 2010). Growth of barnacles after 12 weeks was even enhanced by moderate OA (1030 µatm) as compared to control pCO$_2$ or values exceeding 1930 µatm.

Warming as well as short exposure to pCO$_2$ values of 2140 µatm, positively affected growth in terms of an increase in basal diameter (week 2 and 4; batch culture experiment), but pCO$_2$ values of 1930 and 2870 µatm reduced growth rates (basal diameter as well as DW) under longer exposure (week 10 and 12; flow-through experiment). Increased calcification under increased pCO$_2$ (“compensatory calcification”) was observed earlier in basal parts of shell plates (active growth zone) of the barnacles *A. amphitrite* (McDonald et al. 2009). Further examples exist for echinoderms and oysters (e.g. Wood et al. 2008; Beniash et al. 2010). This increased calcification, however, comes with a cost for the organism possibly taking energy away from other important processes (Wood et al. 2008; Gutowska et al. 2010). Nevertheless, since this effect disappeared at week 4 and was not observed thereafter, acclimatisation to changes in pCO$_2$ seems possible.

Barnacle shells contain <3% organic material (of the total shell mass), while the remaining material is formed of minerals, predominantly calcite (Barnes et al. 1976; Bourget 1987, Findlay et al. 2010a). Although OA is expected to negatively impact calcification processes in marine organisms (Orr et al. 2005, Findlay et al. 2010a), calcification in *A. improvisus* seems not impacted, but barnacles produced noticeably more shell material when exposed to increased temperatures. Crustaceans are known to possess high metabolic rates and are capable of controlling extracellular pH through active ion transport (Whiteley 2011). Furthermore, Whiteley (2011) discusses that calcification in crustaceans is likely to be less affected by OA because of the CaCO$_3$ produced being mostly in the more stable form of calcite rather than the more soluble aragonite, the calcification processes being well isolated from external changes in the surrounding seawater and the use of HCO_3^- rather than CO_3^{2-} for CaCO$_3$ precipitation.

Results of shell-strength measurements further support the observed investment of barnacles' energy into calcification (i.e. shell production). Shell-strength (shear force and breaking resistance) was increased with increasing temperature. A significant effect of pCO_2 was observed for the breaking resistance only with less force required to break shells of barnacles reared under 2140 µatm pCO_2. In an earlier study investigating OA effects on a barnacle species of the same genera, calcification was also increased at the lower base, i.e. the active growth region, while shell-strength at the central parts of the shell plates were compromised pH 7.4 (McDonald et al. 2009).

Overall, results from the field growth measurements as well as from the lab experiments suggest a high tolerance of juveniles of *A. improvisus* to predicted near future OA (Caldeira and Wickett 2005; Orr et al. 2005). Larval stages (nauplii and cyprids) of *A. improvisus* were previously shown to tolerate significant levels of OA up to 3250 µatm (Pansch et al. 2012). Heterotrophic degradation of organic material in deeper waters is directly related to oxygen consumption and CO_2 production (Rabalais et al. 2002; Diaz and Rosenberg 2008) and upwelling events will expose organisms in shallow marine habitats to these corrosive waters. Due to carbonate system kinetics, elevated pCO_2 values will persist at surface waters while oxygen will rapidly equilibrate with the atmosphere. Such processes can be observed for the Kiel Fjord (Thomsen et al. 2010) as well as in many shallow seas worldwide (Blackford and Gilbert 2007; Shim et al. 2007; Feely et al. 2008; Salisbury et al. 2008; Wootton et al. 2008; Miller et al. 2009; Feely et al. 2010) and local organisms might have adapted to cope with these fluctuations. This naturally occurring acidification within Kiel Fjord and barnacles' tolerance to naturally encountered OA highlights upon the importance in evaluating OA experiments in its ecological context. In the course of global change, however, atmospheric CO_2 concentrations will continue to rise (IPCC 2007) and will amplify OA in these coastal waters. River discharges of acidic water can further exacerbate seawater acidification (Salisbury et al. 2008). Model calculations predict maximum pCO_2 values of even 4000 µatm (at a salinity of 20 and a temperature of 10 °C) in estuaries such as the Kiel Fjord (Melzner et al. 2012). Considering these model calculations on possible future OA as well as experimental results obtained herein near future OA might be tolerated by juvenile *A. improvisus* but OA levels up to 4000 µatm will most likely impair barnacle performance in the long term.

We observed reduced growth rates as well as weakening of barnacle shells in the long term under very strong OA. However, in common with recent investigations on barnacles (e.g. McDonald et al. 2009, Findlay et al. 2010a, b), these impacts were subtle and sub-lethal and increased temperature as expected to occur in the future (IPCC 2007) has the potential to mitigate the negative effects of OA (Brennand et al. 2010; Waldbusser et al. 2011; present study). Even in water undersaturated with respect to calcite, where calcification processes are compromised (Orr et al. 2005) and dissolution of the outer shell is likely to occur (Nienhuis et al. 2010), net production of considerable amounts of $CaCO_3$ could be sustained. Community structures are nevertheless susceptible to possible changes in an acidified ocean due to species-specific differences in sensitivities to OA within a community (Hale et al. 2011). As discussed for crustaceans (Whiteley 2011) and possibly valid in a broader context, certain species inhabiting fluctuating environments, such as estuaries and shallow coastal seas are likely to be more tolerant to OA.

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Tables

Table 1: Carbonate system of various shallow benthic habitats within the western Baltic Sea - Waters samples were taken during a ship cruise in September 2009 via scuba diving. In-Situ salinity (S), temperature (T), dissolved inorganic carbon (C_T) and total alkalinity (A_T) measurements as well as calculated pH$_T$, pCO$_2$, $\Omega_{Calcite}$ and $\Omega_{Aragonite}$; undersaturated samples with respect to calcite or aragonite are underlined.

location	coordinates	characterization	depth	S	T	C_T	A_T	pH$_T$	pCO$_2$	Ω_{Ca}	Ω_{Ar}
			[m]		[°C]	[µmol kg^{-1}]	[µmol kg^{-1}]		[µatm]		
Falshöft	54°46,94'N 09°57,32'E	sand, small boulders: dominated by *Zostera marina*, *Ciona intestinalis*, *Mytilus edulis*, *Amphibalanus improvisus*	4	17.9	18.0	1800.2	1924.6	8.05	402.1	2.67	1.61
Schleimünde	54°41,56'N 10°02,08'E	sand, small boulders: dominated by *Fucus* sp, *Ulva* sp. or *Pilayella* sp.	2	18.2	19.0	1897.4	1942.6	7.73	905.0	1.44	0.87
			3	18.0	18.0	1817.7	1937.8	8.04	423.5	2.60	1.56
			5	18.0	15.0	1810.8	1947.3	8.13	328.6	2.84	1.70
			8	18.2	17.0	1834.0	1950.2	8.03	427.2	2.51	1.51
			15	20.4	17.0	2040.8	2044.3	7.55	1423.9	0.99	0.61
Boknis Eck	54°2,92'N 10°01,77'E	boulders: red algae, *Chorda filum, A. improvisus*	4	17.7	18.0	1828.3	1926.3	7.97	504.5	2.23	1.34
Fehmarn NE	54°32,15'N 11°04,62'E	sand, small boulders: dominated by *Fucus* sp., *M. edulis*, *A. improvisus*	4	15.6	17.0	1755.4	1866.8	8.08	381.4	2.49	1.47
Staberhuk (Fehmarn SE)	54°24,10'N 11°17,80'E	sand, big boulders: dominated by *Z. marina*, *M. edulis*, *A. improvisus*	2	15.0	18.0	1789.5	1854.4	7.89	611.0	1.73	1.02
			3	15.0	18.0	1838.1	1860.9	7.70	983.7	1.14	0.68
			3	15.2	18.0	1799.0	1864.0	7.89	618.2	1.73	1.02
			5	15.1	17.0	1800.0	1861.8	7.89	612.2	1.67	0.98
			8	17.0	16.0	1847.4	1877.5	7.73	886.1	1.19	0.71
			11	19.1	17.0	1903.5	1965.7	7.82	731.3	1.64	1.00
			15	19.8	16.0	1932.9	1963.1	7.69	990.7	1.21	0.74
Kellenhusen	54°11,80'N, 11°05,90'E	rocky: largely dominated by *M. edulis*	4.3	15.7	18.0	1843.6	1866.0	7.69	1006.9	1.13	0.67
Salzhaff	54°2,37'N, 11°31,59'E	mud: dominated by *Z. marina, Z. noltii, C. intestinalis*	3.3	13.1	17.0	1802.4	1856.5	7.89	628.1	1.59	0.93

Table 2: Carbonate system of both lab experiments - In-Situ measurements of pH_{NBS}, dissolved inorganic carbon (C_T) and pH_T as well as calculated total alkalinity (A_T), pCO_2, $\Omega_{Calcite}$ and $\Omega_{Aragonite}$ of the batch culture (BC) and the water flow-through (WFT) experiment under different temperature × pCO_2 treatment combinations as well as of the incoming Kiel Fjord water (mean ± s.d.).

			In-situ measurements			calculations			
		air pCO_2 [ppm]	pH_{NBS}	pH_T	C_T [µmol kg^{-1}]	A_T [µmol kg^{-1}]	pCO_2 [µatm]	Ω_{Ca}	Ω_{Ar}
BC	20 °C	388	7.863 ± 0.183	7.795 ± 0.142	1638.8 ± 605.0	1591.2 ± 491.4	664.2 ± 134.0	1.41 ± 0.93	0.84 ± 0.56
		1120	7.664 ± 0.183	7.648 ± 0.148	1673.4 ± 602.0	1622.0 ± 513.5	953.6 ± 83.1	1.04 ± 0.82	0.62 ± 0.5
		4000	7.306 ± 0.181	7.327 ± 0.097	1751.3 ± 568.7	1628.8 ± 444.5	2069.9 ± 139.5	0.50 ± 0.23	0.30 ± 0.14
	24 °C	388	7.906 ± 0.199	7.766 ± 0.130	1651.7 ± 534.2	1640.6 ± 444.8	729.7 ± 114.0	1.67 ± 1.03	1.01 ± 0.63
		1120	7.713 ± 0.180	7.616 ± 0.148	1692.5 ± 602.0	1648.1 ± 513.5	1043.6 ± 100.9	1.25 ± 0.82	0.76 ± 0.50
		4000	7.323 ± 0.190	7.295 ± 0.139	1731.2 ± 616.0	1616.7 ± 501.6	2212.5 ± 49.7	0.60 ± 0.36	0.36 ± 0.22
WFT	20 °C	388	8.082 ± 0.117	7.920 ± 7.079	1906.5 ± 65.4	1998.4 ± 67.0	617.4 ± 101.1	2.14 ± 0.42	1.28 ± 0.25
		1120	7.789 ± 0.109	7.710 ± 0.041	1962.2 ± 88.6	2002.3 ± 89.7	1027.8 ± 92.5	1.36 ± 0.14	0.81 ± 0.09
		2400	7.453 ± 0.099	7.452 ± 0.038	2036.5 ± 69.1	2015.2 ± 72.7	1929.4 ± 126.9	0.77 ± 0.08	0.46 ± 0.05
		4000	7.249 ± 0.106	7.289 ± 0.047	2111.1 ± 78.9	2045.6 ± 86.6	2867.4 ± 222.3	0.55 ± 0.08	0.33 ± 0.05
		Fjord	7.928 ± 0.145	7.735 ± 0.057	2010.7 ± 89.4	2057.9 ± 87.2	1001.3 ± 124.7	1.49 ± 0.07	0.89 ± 0.07

Table 3: Batch culture experiment - Repeated measures ANOVA of size, dry weight and condition index as well as factorial ANOVA of shear force- and breaking resistance of juveniles of *Amphibalanus improvisus* under different temperature (T) × $p\mathrm{CO}_2$ treatment combinations over 56 days (p_{corr} = corrected p values when the assumption of sphericity was violated). Values of $p < 0.05$ are underlined (N=8).

		SS	d.f.	MS	F	p	p_{corr}
size	T	0.6655	1	0.6655	5.8528	0.0200	
	$p\mathrm{CO}_2$	0.2491	2	0.1246	1.0954	0.3438	
	T × $p\mathrm{CO}_2$	0.0152	2	0.0076	0.0668	0.9355	
	Day	861.1982	3	287.0661	9559.5206	0.0000	<0.0001
	Day × T	0.2558	3	0.0853	2.8395	0.0407	0.0739
	Day × $p\mathrm{CO}_2$	0.7549	6	0.1258	4.1898	0.0007	0.0066
	Day × T × $p\mathrm{CO}_2$	0.0178	6	0.0030	0.0987	0.9964	0.9710
dry weight	T	0,000161	1	0,000161	10.3928	0.0025	
	$p\mathrm{CO}_2$	0,000015	2	0,000008	0.4940	0.6139	
	T × $p\mathrm{CO}_2$	0,000010	2	0,000005	0.3156	0.7311	
	Day	0,033556	3	0,011185	685.2831	0.0000	<0.0001
	Day × T	0,000032	3	0,000011	0.6477	0.5859	0.4924
	Day × $p\mathrm{CO}_2$	0,000025	6	0,000004	0.2516	0.9578	0.8702
	Day × T × $p\mathrm{CO}_2$	0,000064	6	0,000011	0.6533	0.6874	0.5924
condition index	T	0.0563	1	0.0563	33.8109	<0.0001	
	$p\mathrm{CO}_2$	0.0037	2	0.0018	1.1012	0.3423	
	T × $p\mathrm{CO}_2$	0.0002	2	0.0001	0.0496	0.9516	
	Day	0.1733	3	0.0578	45.1219	<0.0001	<0.0001
	Day × T	0.0008	3	0.0003	0.2097	0.8895	0.7737
	Day × $p\mathrm{CO}_2$	0.0067	6	0.0011	0.8746	0.5158	0.4690
	Day × T × $p\mathrm{CO}_2$	0.0010	6	0.0002	0.1302	0.9923	0.9552
shear force resistance	T	78414.4285	1	78414.4285	13.0971	0.0004	
	$p\mathrm{CO}_2$	10645.1899	2	5322.5950	0.8890	0.4134	
	T × $p\mathrm{CO}_2$	4313.3458	2	2156.6729	0.3602	0.6982	
breaking resistance	T	403245.5878	1	403245.5878	25.0546	<0.0001	
	$p\mathrm{CO}_2$	104573.4439	2	52286.7220	3.2487	0.0418	
	T × $p\mathrm{CO}_2$	29232.8481	2	14616.4240	0.9082	0.4057	

Table 4: Water flow-through experiment - Repeated measures ANOVA of size, dry weight and condition index as well as factorial ANOVA of shear force- and breaking resistance of juveniles of *Amphibalanus improvisus* under different $p\mathrm{CO}_2$ treatments over 84 days (p_{corr} = corrected p values when the assumption of sphericity was violated). Values of $p < 0.05$ are underlined (N=5).

		SS	d.f.	MS	F	p	p_{corr}
size	$p\mathrm{CO}_2$	4.1532	3	1.3844	3.7389	0.0346	
	Day	908.1749	5	181.6350	2786.8619	0.0000	<0.0001
	Day × $p\mathrm{CO}_2$	4.4284	15	0.2952	4.5298	<0.0001	0.0038
dry weight	$p\mathrm{CO}_2$	0.0016	3	0.0005	4.2818	0.0243	
	Day	0.0589	2	0.0295	220.8668	0.0000	<0.0001
	Day × $p\mathrm{CO}_2$	0.0025	6	0.0004	3.1364	0.0177	0.0529
condition index	$p\mathrm{CO}_2$	0.0121	3	0.0040	0.9142	0.4592	
	Day	0.1504	2	0.0752	19.0521	<0.0001	0.0003
	Day × $p\mathrm{CO}_2$	0.0398	6	0.0066	1.6788	0.1632	0.2057
shear force resistance	$p\mathrm{CO}_2$	29202.1226	3	9734.0409	1.0206	0.3949	
breaking resistance	$p\mathrm{CO}_2$	16526.7531	3	5508.9177	0.2532	0.8587	

Figures

Figure 1: Illustration of *Amphibalanus improvisus* describing the methods in determining the force required to dislodge the barnacles from the settlement panel (shear force) as well as the force required to break the barnacle shell (breaking force).

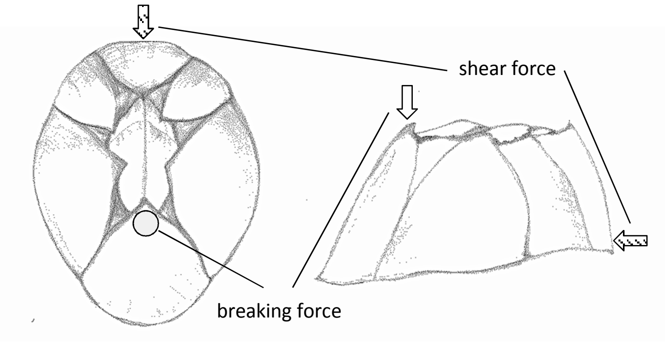


Figure 2: Mean barnacle growth of two cohorts of juveniles of *Amphibalanus improvisus* in the field: cohort-I (black circles) settled in July 2010 and was used in the "batch culture experiment"; cohort-II (grey circles) settled in October 2010 and was used in the "water flow-through experiment" (means ± 95% CI). Water temperatures at the site for 1 m depth are given from July 2010 to May 2011.

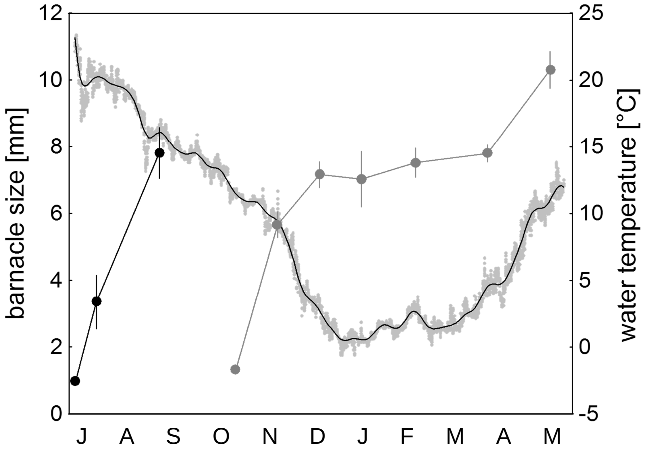


Figure 3: Batch culture experiment - size (**a**), dry weight (**b**) and condition index (**c**) of *Amphibalanus improvisus* individuals over 56 days as well as shear force- and breaking resistance at the end of the experiment (day 62; **d**) under different temperature × $p\mathrm{CO}_2$ treatment combinations (means ± 95% CI; N=8). Asterisks represent significant differences between treatments within a sampling day (according to Fisher's LSD test at $p < 0.05$).

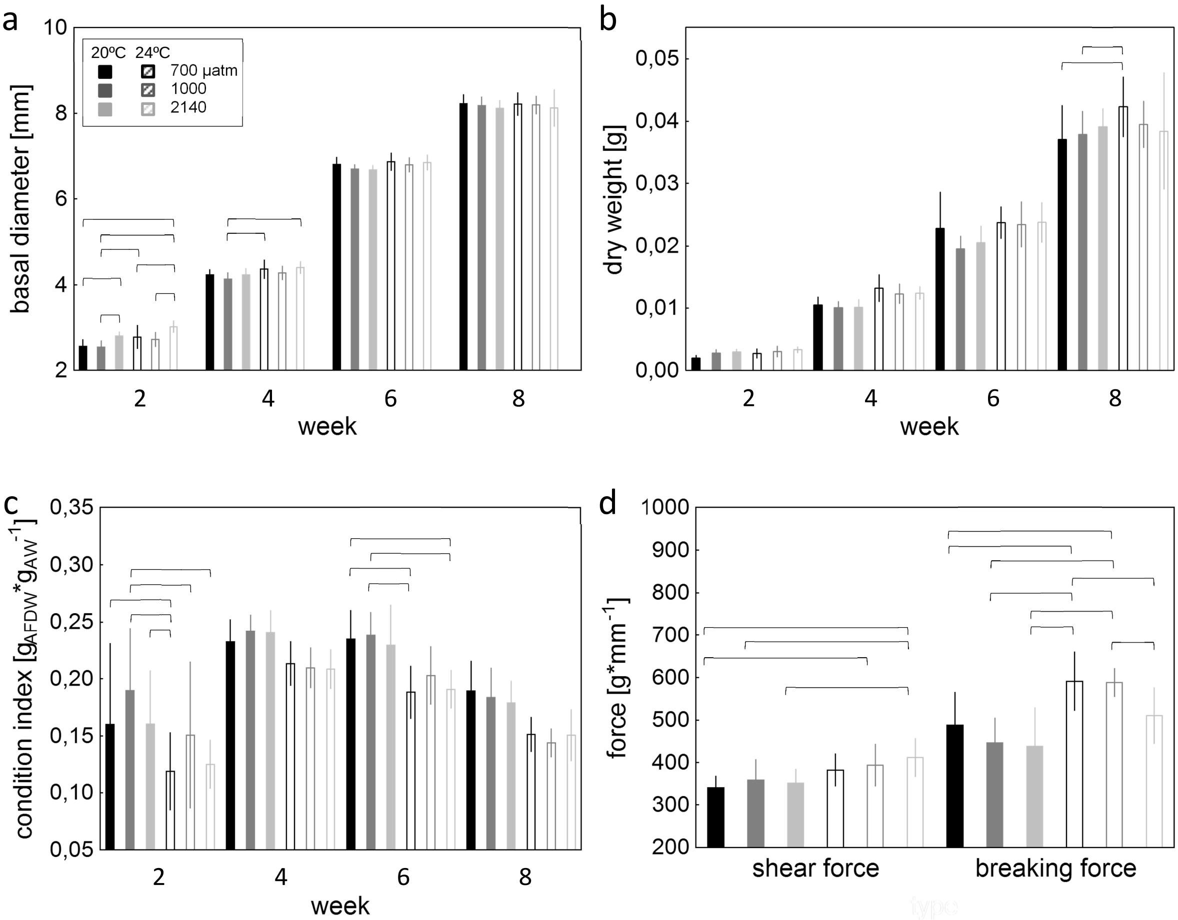
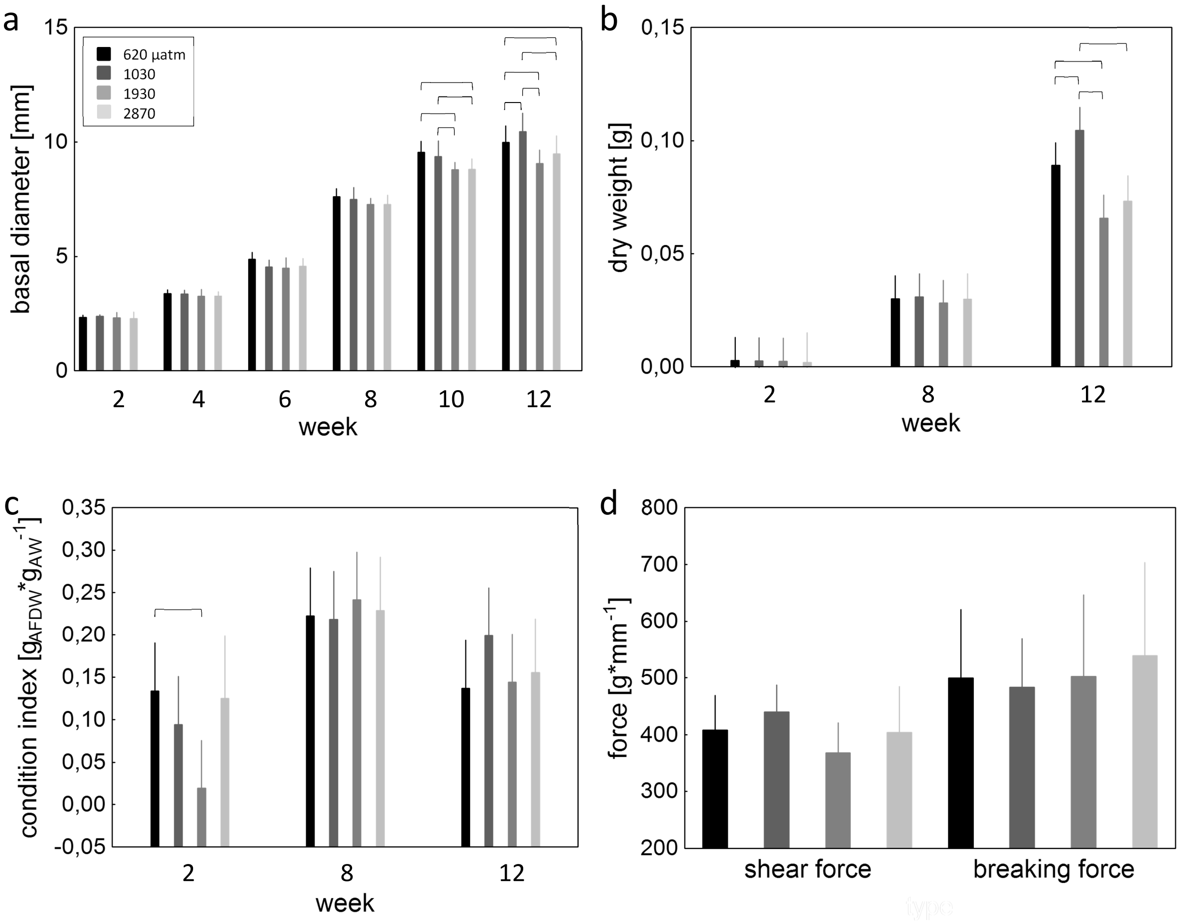


Figure 4: Water flow-through experiment - size (**a**), dry weight (**b**) and condition index (**c**) of *Amphibalanus improvisus* individuals over 85 days as well as shear force- and breaking resistance at the end of the experiment (day 86; **d**) under different $p\text{CO}_2$ treatments (means ± 95% CI; N=5). Asterisks represent significant differences between treatments within a sampling day (according to Fisher's LSD test at $p < 0.05$).



Publication IV – Juvenile development of the Kiel vs. the Tjärnö population

Do habitat traits and food availability modulate the impact of ocean acidification on juvenile Amphibalanus improvisus?

Do habitat traits and food availability modulate the impact of ocean acidification on juvenile *Amphibalanus improvisus*?

Christian Pansch[1*], Iris Schaub[2], Jonathan N Havenhand[3], Martin Wahl[1]

[1]Department of Marine Ecology, GEOMAR - Helmholtz Centre for Ocean Research Kiel, 24105 Kiel, Germany
[2]Institute of Biological Sciences, Marine Biology, University of Rostock, 18059 Rostock, Germany
[3]Department of Biological & Environmental Sciences – Tjärnö, University of Gothenburg, Tjärnö, 45296 Strömstad, Sweden

Statement of authorship: CP and IS contributed equally to this work. CP, IS and MW designed the study, CP and IS collected and analysed the data and wrote the manuscript and all authors contributed substantially to revisions

Corresponding author[*]: Tel: +49 431 600 4084
Fax: +49 431 600 1671
Email: cpansch@geomar.de

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Abstract

Energy availability and adaptation are major threads in tolerating ocean acidification (OA) stress. Sufficient food availability in eutrophied habitats can help organisms to overcome OA related stress effects, while organisms from habitats with naturally high fluctuations in pCO_2 might have evolved characteristics that make them more tolerant to future OA than organisms from more stable environments. We investigated effects of diet and pCO_2 (pCO_2 values of nominally 380, 1000 and 3000 µatm) on newly settled *Amphibalanus improvisus* in different barnacle populations: in a 20 weeks experiment in Kiel, Germany and in a 5 weeks experiment in Tjärnö, Sweden. The Kiel Fjord is characterized by strong fluctuations in pCO_2 whereas Tjärnö waters display a more stable pCO_2 environment. Kiel barnacles showed a strong tolerance to all applied pCO_2 levels with respect to survival, growth, reproduction and shell strength as well as to F1 (offspring) larval performance and F1 juvenile growth, regardless of diet. Moulting frequency and dissolution of outer shell material, however, were increased with increasing pCO_2. Tjärnö barnacles compensated for moderate pCO_2 when food conditions were ample but showed reduced growth under food limitation. Severe pCO_2 negatively impacted growth of Tjärnö barnacles in both feeding treatments. Similar patterns were observed for survival rates of Tjärnö barnacles. These results demonstrate a high tolerance of Kiel barnacles to OA, even when considering carry-over effects of adult individuals to their F1 generation. This study further supports the hypothesis that barnacle populations from fluctuating pCO_2 environments are more tolerant to OA than barnacles from more stable pCO_2 habitats. The results show first evidence that energy availability might help barnacles to overcome moderate OA stress.

Introduction

Anthropogenically increased atmospheric CO_2 causes a reduction in oceanic pH – a phenomenon referred to as Ocean Acidification (“OA”; Caldeira and Wickett 2003; Gattuso and Hansson 2011). While oceanic pH decreases at relatively constant rates of ~0.002 units per year (Doney et al. 2009), coastal habitats, have been recently described to differ substantially from open ocean conditions. Not only absolute mean values and annual or daily fluctuations (e.g. Blackford and Gilbert 2007; Shim et al. 2007; Feely et al. 2008; Salisbury et al. 2008; Wootton et al. 2008; Miller et al. 2009; Feely et al. 2010) but even future predictions differs considerably from open ocean norms (e.g. Wootton et al. 2008; Feely et al. 2010; Melzner et al. 2012).

Processes such as eutrophication, stratification, degradation or upwelling can amplify atmospheric driven OA and, already today, may produce more acidified conditions in coastal habitats (Wootton et al. 2008; Miller et al. 2009; Feely et al. 2010; Thomsen et al. 2010) than what has been predicted to occur for open ocean waters by the end of this century or even later (Caldeira and Wickett 2003). The Western Baltic Sea and especially coastal bays such as the Kiel Fjord or the Eckernförde Bay represent such a habitats where annual mean $p\mathrm{CO}_2$ concentrations of about 800 µatm prevail already today (Thomsen et al. 2010; Thomsen et al. under review in Nature Climate Change) with peak values of up to 2300 µatm during upwelling events in late summer and autumn (Thomsen et al. 2010; Vincent Saderne pers. commun.). Furthermore, brackish habitats such as the Baltic Sea exhibit generally lower salinity values and consequently a lower alkalinity (Hjalmarsson et al. 2008) and thus, naturally lower $CaCO_3$ saturation states (Ω) compared to open ocean waters. It may be instructive to study how organisms cope with this naturally acidified environment in marginal seas as these may constitute a window to future oceanic conditions.

The impacts of stressors such as OA on organisms, however, are not simple or uniform. They are highly species specific (Kroeker et al. 2010; Whiteley 2011) and may be modulated by numerous factors such as the distance between the stressing variable and the organism’s optimum (stress intensity), the duration and fluctuations of the stressor (habitat traits), the stress history of the individual, the existence of other synchronous biotic or abiotic stressors, the existence of compensating mechanisms, their energy demand and/or the energy availability in the habitat.

Thus, organisms, which live in stable but food-depleted environments such as the deep-sea or polar regions might be sensitive to OA, while organisms from fluctuating and, in many cases, food-enriched environments, such as estuaries and shallow coastal regions, tend to be more tolerant to OA (Dupont et al. 2010; Whiteley 2011). A barnacle (*Semibalanus balanoides*) population from Spitsbergen showed reduced growth under OA (Findlay et al. 2010b), while a population of the same species from the probably more variable and energy rich environments along the southern British coast was tolerant to OA (Findlay et al. 2009, 2010a, c). Such habitat specific responses of organisms could be a consequence of long-term acclimations or even genetic adaptations in response to the nature of a particular habitat with respect to environmental stressors such as OA.

An organism's tolerance to a certain stressor largely depends on the availability of compensatory mechanisms and the energy availability to compensate stress. Thus energy availability can be considered as a major thread in tolerating future OA stress (e.g. Melzner et al. 2011). In field studies, mussels withstood naturally occurring OA stress in eutrophied food-rich habitats but suffered from naturally occurring OA stress in oligotrophic food-depleted areas (Hall-Spencer et al. 2008; Thomsen et al. under review in Nature Climate Change).

Barnacles are important ecosystem components (e.g. Berntsson and Jonsson 2003). Nonetheless, only few studies investigated the impact of OA on barnacles (Findlay et al. 2009; McDonald et al. 2009; Findlay et al. 2010a, b; Pansch et al. 2012). *Semibalanus balanoides* as well as *Amphibalanus amphitrite* showed an increase in calcification under acidified conditions, which was interpreted as a compensatory response to higher dissolution of exposed shell material (Findlay et al. 2009; McDonald et al. 2009). Only *S. balanoides* showed a reduction in growth under OA, interpreted as a reallocation of available energy from growth to shell maintenance (Findlay et al. 2010b). This could be due to physiological differences between the two species but could also be a result of the different food concentrations used in the respective studies (2×10^6 cells ml^{-1} daily for *A. amphitrite* vs. 1.5×10^4 cells ml^{-1} every second day for *S. balanoides*).

Active ion regulation is an energy demanding process, which increases during acidified conditions (Melzner et al. 2009; Whiteley 2011). In addition, dissolution of outer shell material increases with decreasing saturation state of calcium carbonate, which has to be compensated for by an increase in calcification rate to maintain shell stability (McDonald et al. 2009). While *A. improvisus* shows no or only weak responses to medium term OA under ample food conditions (Pansch et al. under review in Marine Biology), under food limitation, increased metabolic rates and shifts of energy budgets towards ion regulation could reduce the energy available for growth or other energy demanding processes as has been demonstrated for other invertebrates (Whiteley 2011; Thomsen et al. under review in Nature Climate Change).

Despite the importance of considering carry-over effects when predicting adaptation capacities to OA (Dupont et al. 2010; Sanford and Kelly 2011) few OA studies have considered links between life-history stages or between adults and their offspring when determining a species' response to OA (but see Kurihara et al. 2008; Egilsdottir et al. 2009; Parker et al. 2009, 2010, 2012). Carry-over effects from larval stages to post-larvae (juveniles) in response to stressors such as temperature and salinity, in contrast to OA, have been well investigated for barnacles (Thiyagarajan et al. 2002, 2003, 2007). In one of the rare studies on carry-over effects of OA, McDonald et al. (2009) examined the effects of OA on larval development and subsequent settlement and juvenile growth as well as on egg production in *A. amphitrite* with no signs of any carry-over effects from one to the other stage. In another study on *S. balanoides*, embryonic development was delayed under OA indicating maternal effects on barnacle reproduction and thus possible negative effects on their overall fitness (Findlay et al. 2009). Both studies, however, neglect possible important maternal effects on the performance of larvae or post-larvae.

In the present study, we examined the performance (survival, growth, moulting frequency, shell strength) of the barnacle *A. improvisus* during the developmental phase from juvenile to adult barnacles. We also assessed the reproductive output under food limitations (and thus energy restriction) of barnacles, which had grown to maturity und OA conditions as compared to actual in-situ conditions. We investigated possible carry-over effects of OA from parents to their F1 generation (offspring) with respect to larval development and to juvenile growth. Finally, we compared two populations of *A. improvisus* with respect to OA sensitivity; one from the fluctuating western Baltic Sea (Kiel Fjord) and one from the eastern Skagerrak (Tjärnö Archipelago), a presumably more stable environment with more oceanic conditions and pH values rarely dropping below 8.0 (Larsson 2010).

We hypothesized (i) that food availability will affect the barnacles' capacity to cope with OA stress, (ii) that a possible stress impact may be carried over to subsequent life history stages, and (iii) that barnacle populations from fluctuating $p\mathrm{CO}_2$ environments are more tolerant to OA than barnacles from more stable $p\mathrm{CO}_2$ habitats.

Materials and methods

All experiments were conducted in constant temperature rooms at the GEOMAR in Kiel, Germany and at the Sven Lovén Centre for Marine Sciences in Tjärnö, Sweden during summer and autumn 2011. Juveniles of the barnacle *Amphibalanus improvisus* were collected by exposing transparent settlement panels (Perspex®, 9 × 9 cm) in the subtidal zone (1.5 m depth) in the inner Kiel Fjord (54°19.5'N, 10°09.0'E) in mid-June and in the Tjärnö Archipelago (58°52.5'N, 11°08.1'E) in mid-August. Panels were left in the water for two weeks with barnacles being able to only settle on the bottom side (the upward face of the panel being covered by a grey PVC panel during the settlement phase). In order to standardize barnacle density to 26 individuals per panel, at the end of the settlement phase surplus barnacles were gently removed. Two settlement panels each were distributed to aquaria of the different feeding × acidification treatment combinations. Each treatment combination was replicated 8 times for the Kiel experiment and 6 times for the Tjärnö experiment. A set of panels was left in the field (Kiel and Tjärnö) in order to observe natural growth rates.

Feeding and acidification treatment levels

Freshly settled barnacles were fed daily with a 1:1 mixture of *Chaetoceros calcitrans* and *Skeletonema costatum*. From week three, barnacles were additionally fed with two day old brine shrimp nauplii (*Artemia salina*). From week five onward only brine shrimps were fed daily or bi-daily. In biweekly intervals, food concentrations were increased and the number of barnacles was reduced in order to compensate for barnacle growth and increasing food consumption. A ratio of 5:1 (high-food:low-food, cell and/or nauplii numbers) between the two feeding levels was maintained during the entire experiment (Table 1). Diatom algae were cultured in filtered (0.2 µm) and autoclaved seawater enriched with f/2 medium. Algal densities were determined using a haemocytometer (Neubauer, Marienfeld, Germany) under the microscope. *Artemia* were cultured in filtered (0.2 µm) seawater in aerated 1 l plastic bottles and densities were determined under a stereomicroscope. Following the addition of food to the experimental aquaria the water flow in was stopped overnight.

The three acidification levels of nominally 380, 1000 and 3000 µatm were chosen according to the high natural variability of $p\mathrm{CO}_2$ in coastal seas and taking predicted future scenarios into account (Thomsen et al. 2010; Wahl et al. 2010; Melzner et al. 2012). The different acidification levels for the Kiel experiment were achieved by direct aeration of the experimental aquaria with either ambient air or pre-mixed gas with $p\mathrm{CO}_2$ concentrations of 1120 or 4000 ppm. Acidification levels for the Tjärnö experiment were achieved by direct aeration of the experimental aquaria with either ambient air or air, which was enriched with pure, gaseous CO_2 controlled by computerized pH controllers (NBS scale, resolution: 0.01 units, Aqua Medic® GmbH, Germany, IKS® Computer Systeme GmbH, Germany). Target pH values for the Tjärnö experiment were determined previously by aerating water samples with either ambient air or with industrial pressurized air of fixed $p\mathrm{CO}_2$ concentrations of 977 and 3000 ppm (AGA Gas AB, Enköping, Sweden). The achieved pH levels were then used to set the respective pH controllers.

Experimental setup and carbonate chemistry measurements

Both experiments were run in 3.7 l covered plastic aquaria connected to a flow-through water system. Seawater was pumped directly from the Kiel Fjord or the Tjärnö Archipelago into temperature rooms, adjusted to a constant water temperature of 20 °C (Aqua Medic®, Germany) and pumped into header tanks from which each single aquarium was supplied with a constant water flow of ~1.5 l h^{-1}. In the Kiel experiment juveniles of an initial mean size (± s.d.) of 0.794 ± 0.079 mm were maintained under these treatment combinations for 20 weeks. In the Tjärnö experiment, juveniles of an initial mean size of 1.205 ± 0.100 mm were maintained for 5 weeks. We used a 12/12 h day/night cycle.

Temperature and pH_{NBS} (NBS scale) measurements were done twice per week in four random replicates in Kiel and in all six replicates in Tjärnö using a WTW® 330i pH meter equipped with a SenTix® 81 pH electrode. Salinity measurements were done with a WTW® Cond 340i equipped with a TetraCon® 325 electrode during the Kiel experiment and with an YSI 30® during the Tjärnö experiment. During the Kiel experiment, additional water samples for C_T (total dissolved inorganic carbon) and pH_T (Total scale) measurements were taken once per week from two random replicates. C_T was measured coulometrically (AIRICA® C_T analyser; Marianda, Kiel, Germany) and pH_T was determined with a 826-Metrohm® mobile pH meter equipped with a Methrom® 6.0262.100 electrode using Tris/HCl and AMP/HCl seawater buffers (Dickson et al. 2007). Measurements were corrected using DICKSON seawater standard as reference material (Dickson et al. 2003). During the Tjärnö experiment, additional water samples for $p\mathrm{CO}_2$ determination and pH_T measurements were taken twice per week in one random replicate. pH_T was determined with a WTW® 330i pH meter equipped with a SenTix® 81 pH electrode using Tris/HCl and AMP/HCl seawater buffers. Measurements were conducted in fresh samples and in the samples equilibrated with pressurized air of fixed $p\mathrm{CO}_2$ concentrations of 977 and 3000 ppm (AGA Gas AB, Enköping, Sweden). All other parameters ($p\mathrm{CO}_2$/DIC, A_T, $\Omega_{\mathrm{Aragonite}}$ and $\Omega_{\mathrm{Calcite}}$) were calculated using the CO_2SYS macro (Pierrot et al. 2006) with dissociation constants (K_1 and K_2) according to Millero et al. (2006) using KHSO_4 dissociation constant after Dickson (1990). Additional seawater measurements were done weekly in freshly taken field samples as mentioned previously.

Response variables

In the Kiel experiment we continuously monitored mortality, basal diameter, dry weight (DW; including body and shell mass), ash weight (AW; = shell mass), the condition index (CI; = body to shell ratio). At the end of the experiment we assessed moulting activity, larval release, net-calcification rates and breaking resistance of barnacles as well as F1 larval settlement and F1 juvenile growth. We continuously monitored mortality, basal diameter, DW, AW and CI of barnacles for the Tjärnö experiment. From measurements on single barnacles (“basal diameter”, “DW”, “AW”, “CI” and “breaking resistance”) means were calculated for single aquaria (i.e. representing one replicate).

Mortality, growth and CI: Overall mortality was assessed by calculating a mean daily mortality from all barnacles dying during the entire experiment. To determine barnacle growth by means of an increase in maximum basal-plate diameter, images from the backside of each transparent settlement panels were taken using a digital camera (CANON EOS, EFS 18-55mm) and the maximum basal plate diameter of each single barnacle was measured using image analysis software (ImageJ 1.43u). For DW measurements, six barnacles were randomly scraped from the panels at each sampling day (Kiel: week 2, 6, 12, 20; Tjärnö: week 2, 4, 5) and frozen at -20 °C. At the end of the experiment, barnacles were dried at 80 °C (MMM® Ecocell) for 24 h and weighed (Sartorius®, ± 0.1 mg). The dried samples were additionally burned for 24 h at 500 °C (Nabertherm® B150) and the remaining inorganic material (AW) was weighed. We calculated ash-free dry weight (AFDW; = body mass) by subtracting AW from DW. The CI for juvenile barnacles was calculated by dividing the AFDW by the AW.

Moulting and reproduction: To determine moulting rates and rates of larval release, filters (90 µm) were placed for 12 h at the outflow of the experimental aquaria in weeks 9 to 12. This was done in five randomly chosen replicates from each feeding × acidification treatment combination. Nauplius larvae as well as the exuviae from the filters were counted. Data were standardized to one hour and one barnacle. Subsequently the relative production of exuviae or larvae contributed by each replicate aquarium of a given treatment combination was calculated and will be in the following be given as “relative moulting rate” or “relative reproductive output”.

Net-calcification rates: To determine net-calcification rates of barnacles in the Kiel experiment, settlement panels from six randomly chosen replicates from each feeding × acidification treatment combination were cleaned from epibionts at the end of the experiment (week 20) and placed into sealed 2 l plastic aquaria containing 1.7 l filtered (0.2 µm) seawater. The aquaria were aerated with the respective treatment $p\mathrm{CO}_2$ concentrations. Water samples were taken at the beginning and at the end of 36 h of incubation. A_T was analysed by potentiometric titration (VINDTA® autoanalyser).

Measurements were corrected using DICKSON seawater standard as reference (Dickson et al. 2003). Aquaria containing filtered (0.2 µm) seawater but no panels with barnacles served as controls. Given the fact that the precipitation of 1 mole of $CaCO_3$ consumes 2 moles of HCO_3^- and therefore decreases A_T by 2 equivalents, net-calcification rates (G) "per g DW of barnacles" (in µmol $CaCO_3 \times g\ DW^{-1} \times h^{-1}$) were estimated using the alkalinity anomaly technique (Smith and Key 1975; Gazeau et al. 2007; Langdon et al. 2011). We used the equation: $G = -\Delta A_T \times 2^{-1}$ where ΔA_T is the variation of A_T during incubation.

Scanning electron microscopy: To examine the morphology of barnacle shells, random barnacle individuals were gently removed from their settlement panels in week 16 and week 18 from each feeding × acidification treatment combination and dried at 80 °C (MMM® Ecocell) for 24 h. Barnacles were coated with gold-palladium and pictures of the whole barnacle as well as of specific areas of the barnacle shells were taken using Scanning Electron Microscopy (SEM; Zeiss® DSM 940).

Shell strength: To determine barnacle shell strength, the force required to break barnacle shells was determined with a TAXT2i texture analyser (Stable Micro Systems®, 25-1 measuring cell) from random barnacle individuals in week 12 and at the end of the experiment. A cylinder of 2 mm diameter pushed slowly onto the rostrum/rostrumlateral-plate (Fig. 5) with a speed of 1.0 mm × s^{-1} (Pansch et al. under review in Marine Biology). The maximum force required to break the shell was determined (Texture Expert Exceed 2.64; see also McDonald et al. 2009). The breaking force was standardized by barnacle size (basal diameter) given as "g × mm^{-1}" (Appelhans et al. accepted in Marine Ecology-Progress Series; Pansch et al. under review in Marine Biology).

F1 larvae and juveniles: To determine the performance of larvae released by barnacles maintained under the different pCO_2 treatment combinations, nauplius-II larvae were collected on filters (90 µm) over night from six random replicates from each feeding × acidification treatment combination in week 14 of the adult experiment. Larvae were reared at the respective pCO_2 concentrations (i.e. pCO_2 concentrations from the adults as mentioned above) in 250 ml glass bottles filled with 200 ml filtered (0.2 µm) seawater at a constant temperature of 20 °C (Aqua Medic®, Germany) and a concentration of one larva per ml seawater. Larvae were fed bi-daily day with marine diatoms (1:1 mixture of *Chaetoceros calcitrans* and *Skeletonema costatum*, 2×10^5 cells per ml; Nasrolahi et al. 2007) irrespective of the feeding treatment their parents experienced. A water exchange was performed every second day with seawater at the respective pCO_2. Measurements of

pH_{NBS} and temperature were conducted every second or third day in three random replicates from each feeding (adults) × acidification treatment combination and additional water samples for salinity, C_T and pH_T analyses were taken weekly in two random replicates from each feeding (adults) × acidification treatment combination. Water samples were measured and evaluated as described above. Carbonate system values were calculated as means over the adults' feeding treatments (Table 2). Nauplius development is comprised of six nauplius stages and a non-feeding cypris stage (Jones and Crisp 1954). Settlement success of cyprids on the bottle walls was estimated after 16 days as a measure of the overall larval fitness. Growth of F1 juveniles was determined by digital photography (CANON EOS, EFS 18-55mm) of basal plates and image analysis (ImageJ 1.43u) as described above. Pictures were taken on day 34 and day 50 post larval incubation. Juvenile growth is shown as % increase of basal diameter between day 34 and day 50.

Field controls: Natural barnacle growth in the field was assessed by exposing settlement panels to the Kiel Fjord or the Tjärnö Archipelago in 1.5 meter depth at the starting day of the respective lab experiment with sampling procedures as described for the lab experiments (basal diameter, DW, AW, CI and breaking resistance). Newly settled barnacles on the panels in the field were regularly removed to allow competition-free growth of the monitored individuals. Temperature (HOBO® Onset Computer) and salinity (CTD-logger STAR-ODDI®, Island) at the site of the respective field controls were continuously monitored and once per week the carbonate system variables were determined from fresh water samples (for detailed description see above).

Statistical analysis

In both experiments a fully crossed two-factorial design was used with feeding (2 levels) and acidification (3 levels) as the fixed factors. We tested for normality with the Shapiro-Wilk's W-test. Non-normal data were transformed to normality prior to the use of parametric statistics. Percentage data were square-root arcsine transformed. We tested for homogeneous variances using Levene's test. If one of the assumptions could not be verified, we refrained from applying parametric statistical procedures and employed permutation-based multivariate analysis of variance (PERMANOVA; Anderson et al. 2008; Clarke and Gorley 2006).

To analyse breaking resistance, net-calcification rates, larval settlement and juvenile growth from the Kiel population as well as mortality from the Tjärnö population, we used a two-factorial ANOVA (feeding × acidification). Kiel population mortality was analysed with a PERMANOVA (feeding × acidification) based on 9999 permutations and the Euclidean distance matrix. Data from diameter length, DW, AW, CI, moults and larval release measurements were analysed using Repeated Measures PERMANOVA (RM-PERMANOVA; feeding × acidification × time) based on 9999 permutations and Bray Curtis similarity (moults and larval release) and Euclidean distance matrices (all others). We used Fisher’s least significant difference (LSD) tests or PERMANOVA pair-wise tests for multiple comparisons or comparisons within a factor, following ANOVA or PERMANOVA, respectively. All statistics were performed using the software STATISTICA 8.0 and the PERMANOVA+ 1.0.2 add-on for PRIMER 6.1.12 (Anderson et al. 2008).

Results

Water temperature and salinity as well as measurements of the carbonate system parameters from both lab experiments as well as from the respective field-sites are represented in Table 2 and in Fig. 1. Barnacles growing in the field controls experienced water temperatures between 8.6 and 21.3 °C (mean of 16.7 °C) in the Kiel Fjord and between 15.1 and 19.8 °C (mean of 17.3 °C) in the Tjärnö Archipelago over the experimental period of 20 and 5 weeks, respectively (Table 2; Fig. 1a). Salinity ranged between 11.1 and 19.9 (mean salinity of 16.8) in Kiel and between 12.6 and 27.5 (mean salinity of 20.9) in Tjärnö (Table 2; Fig. 1b). Barnacles in the field experienced pH_{NBS} values between 7.53 and 8.36 (mean of 7.91) in Kiel and between 8.02 and 8.29 (mean of 8.16) in Tjärnö (Table 2; Fig. 1c) while the $p\mathrm{CO}_2$ values fluctuated between 286.4 and 1351.1 µatm (mean of 780.4 µatm) and between 345.5 and 519.7 µatm (mean of 401.5 µatm), respectively (Table 2; Fig. 1d). Even very shallow waters within Kiel Fjord exhibited calcite saturation states down to $\Omega_{Ca} = 1.02$ while mean saturation over the 20 weeks was $\Omega_{Ca} = 1.89$ (Table 2). Mean calcite saturation state within the Tjärnö Archipelago was $\Omega_{Ca} = 3.13$ over the experimental period of 5 weeks (Table 2). Seawater was undersaturated with respect to calcite in all treatments with $p\mathrm{CO}_2$ values of nominally 3000 µatm (Table 2).

Kiel experiment

Feeding (interacting with time) significantly affected barnacle growth (diameter, DW, AW) and CI in the Kiel experiment but acidification showed no statistically significant effects (Table 3; Fig. 2). Barnacles grew faster under the high-food compared to the low-food treatment and were 52% larger (mean diameter over the acidification treatments) at the end of the experiment (PERMANOVA pair-wise tests; Fig. 2a-c). The CI was significantly higher under high-food over the 20 weeks (PERMANOVA pair-wise tests; Fig. 2d). The respective field controls showed similar growth rates (diameter, DW and AW) as were obtained in the high-food treatments (Fig. 2a-c). The CI of field control barnacles ranged between the CI of barnacles from the high- and the low-food treatments and decreased over time (Fig. 2d).

Mortality of Kiel barnacles was not significantly affected by either of the two factors (Table 4; Fig. 3a). Moulting was unaffected by feeding but was significantly elevated by increasing $p\mathrm{CO}_2$, irrespective of the feeding treatment (PERMANOVA pair-wise tests; Table 4; Fig. 3b). Barnacles under high-food released significantly more larvae than barnacles under low-food, irrespective of the acidification treatment, which had no effect on larva production (PERMANOVA pair-wise tests; Table 4; Fig. 3c). The force required to break the barnacle shells tested in week 12 and in week 20 was significantly higher under high-food compared to low-food, but was unaffected by acidification (Fisher’s LSD tests; Table 5; Fig. 3d). The field controls showed similar mean resistance to breakage as the high-food × 380 µatm $p\mathrm{CO}_2$ treatment in week 12 (Fig. 3d). There are no data available for week 20 because of a loss of the field sampling panels (Fig. 3d).

The net-calcification rate was significantly reduced with increasing $p\mathrm{CO}_2$ but was unaffected by the feeding treatment (Fisher’s LSD tests; Table 5; Fig. 4). Only the low-food and nominally 3000 µatm $p\mathrm{CO}_2$ treatment had individuals suffering from net dissolution but mean net-calcification rates were always positive, irrespective of the feeding or acidification treatment (Fig. 4). SEM photographs of barnacles at week 19 illustrate the degree of dissolution of outer shell material under elevated $p\mathrm{CO}_2$ (Fig. 5). Barnacles kept under high-food seemed to be more affected than barnacles kept under low-food (Fig. 5).

Water temperature and salinity as well as measurements of the carbonate system parameters from the F1 experiment are represented in Table 2. The overall settlement of F1 larvae and the growth of F1 juveniles were not significantly affected by either of the factors nor was there a significant interaction between both factors (Table 5; Fig. 6a, b).

Tjärnö experiment

Barnacles grew significantly faster under the high-food compared to the low-food treatment and were 25% larger (mean diameter over the acidification treatments) at the end of the experiment (PERMANOVA pair-wise tests; Table 6; Fig. 7a). In week 2 and 4, barnacles at nominally 3000 µatm were significantly smaller than those at 380 µatm pCO$_2$ treatment (by 12 and 18% in week 2 and 4, respectively) under low-food and to those at the nominally 1000 µatm pCO$_2$ treatment (by 15% in week 2 and 4) under high-food (PERMANOVA pair-wise tests; Fig. 7a). After 4 weeks, barnacle size was reduced by 7% under pCO$_2$ values of nominally 1000 µatm in the low-food treatment, however, by week 5, all significant acidification effects on growth had disappeared (PERMANOVA pair-wise tests; Fig. 7a). Barnacles grew faster (DW and AW) under the high-food treatment from week 2 onward but were unaffected by acidification (PERMANOVA pair-wise tests; Table 6; Fig. 7b, c). Barnacles in week 4 had a higher CI under the high-food treatment but were not affected in this regard by acidification (PERMANOVA pair-wise tests; Table 6; Fig. 7d). The respective field controls showed higher growth as well as a higher CI compared to all treatment combination in the laboratory over the experimental period; there are, however no DW, AW and CI data available for week 2 and week 4 (Fig. 2).

Mortality was not affected by the feeding level but increased by 79% under a pCO$_2$ of nominally 3000 µatm compared to 380 µatm under low-food and by 91% higher mortality under a pCO$_2$ of nominally 3000 µatm compared to 380 µatm under high-food (PERMANOVA pair-wise tests; Table 5, Fig. 8).

Discussion

In-situ measurements revealed the Kiel Fjord to be a highly variable habitat with respect to carbonate chemistry as we have reported earlier (Thomsen et al. 2010; Pansch et al. 2012). Towards the end of the experimental period, shallow waters within Kiel Fjord showed calcite saturation states as low as 1, being on the threshold to be corrosive conditions for the calcite shells of barnacles (Barnes et al. 1976; Bourget 1987, Findlay et al. 2010a). Nevertheless, calcifiers such as *A. improvisus* as well as *Mytilus edulis* are present and can even dominate benthic hardbottom communities in Kiel Fjord throughout the year (Dürr and Wahl 2004; Thomsen et al. 2010). Over the experimental period (5 weeks of the Tjärnö experiment) the Tjärnö Archipelago showed to be less variable with respect to carbonate chemistry. Although long-term data sets are not available at this point, this has been reported in earlier studies for this habitat (Larsson 2010) and was also confirmed by Jon Havenhand et al. (pers. commun.).

Kiel Fjord barnacles from a temporarily acidified habitat

Kiel Fjord barnacles showed little sensitivity to acidification. Not even $p\mathrm{CO_2}$ values of nominally 3000 µatm impacted *A. improvisus* growth (diameter, DW and AW) or CI in neither of the feeding treatments. In contrast, food availability did affect barnacle's performance. At the elevated feeding level, barnacles grew at similar rates as in the field, which was about 50% faster than at the reduced feeding level in the lab (0.09 versus 0.06 mm of daily basal diameter increase). With ample food, they also invested more into body growth relative to shell growth. The CI of barnacles kept under OA was not investigated to date but the Pacific oyster *Crassostrea gigas* suffered from OA (pH 7.7) showing a decreased CI by 20%, indicative for reduced growth efficiency due to a likely shift in energy budget (Lannig et al. 2010). Lab growth rates similar to our low-food treatment were reported by McDonald et al. (2009), for the barnacle *Amphibalanus amphitrite* (~0.07 mm per day). Growth rates of the studies on *Elminius modestus* and *Semibalanus balanoides* are not directly comparable because of differences in size measurements (basal diameter versus diameter of the operculum; Findlay et al. 2010a, b). Although Kiel Fjord water conditions were close to undersaturation with respect to calcite during the second half of the 20 week experimental period, both in the lab and in situ, *A. improvisus* showed high daily growth rates of up to 0.09 mm basal diameter.

As another indication for low stress even under strong acidification conditions, mean cumulative barnacle mortality was similarly low in all treatment combinations of the Kiel experiment (<5%; see also Pansch et al. under review in Marine Biology). Interestingly, OA caused mortality of *Semibalanus balanoides* seems to depend on location and season being higher in winter than in summer and in Spitsbergen than on the British coasts (Findlay et al. 2009; Findlay et al. 2010a, b, c). At the moment it is not clear what caused these differences in OA sensitivity and whether we would have found an increased OA driven mortality in seasons other than summer.

Moulting is an essential element in crustacean growth and cirripedia have been reported to moult every 2-3 days (Costlow and Bookhout 1957). In the present study, we observed a tendency for moulting events to increase almost linearly with seawater $p\mathrm{CO_2}$ but not with food availability. Increasing moulting frequency was observed in the shrimp *Palaemon pacificus* under a $p\mathrm{CO_2}$ of 1000 µatm but was reduced under a $p\mathrm{CO_2}$ of 1900 µatm (Kurihara et al. 2008), which was interpreted by the authors as a sequence of increase in metabolic activity and compensatory response to acid base disturbance. In our study, moulting frequency did not relate to growth or CI and for the moment we cannot explain this response pattern.

Since body size and fecundity are strongly correlated in crustaceans (Kurihara et al. 2008 and references therein), any effects on growth should affect the onset of reproduction. In the present study, barnacles in the lab (high-food) as well as in the field reached reproductive size (McDonald et al. 2009) within the first two months of the experiment. Reproductive output was favoured by high-food but unaffected by OA. Larval release in other crustaceans such as copepods was also not impacted by OA (Kurihara 2008; Mayor et al. 2007) supporting these findings. Out of three different barnacle species (*Chthamalus fissus, Balanus glandula, Tetraclita squamosa rubescens*), only *C. fissus* was capable of regulating reproduction (in form of larval output) in dependency of the actual food availability (Hines 1978). Thus, food availability may have impacted reproduction indirectly via growth rates or directly via energy supply to the gonads. While in *Semibalanus balanoides* OA can delay the embryonic development (Findlay et al. 2009) we did not observe this response in the present study.

Shell dissolution in response to OA has been reported for various marine species (Marshall et al. 2008; Ries et al. 2009; Nienhuis et al. 2010; Thomsen et al. 2010; Ries 2011). Here we show that net-calcification (Langdon et al. 2011) of *A. improvisus* was strongly reduced by increasing $p\mathrm{CO}_2$ (irrespective of diet). Nevertheless, growth rates were maintained even in undersaturated water. The negative effect on reduced net calcification may, thus, be mainly attributable to dissolution of outer shell material. Undersaturation with respect to calcite reigned under the 3000 but not under the 1000 µatm $p\mathrm{CO}_2$ treatment. Severe corrosion was observed under nominally 3000 µatm $p\mathrm{CO}_2$ and was negligible under nominally 1000 µatm $p\mathrm{CO}_2$. The latter, surprisingly indicated corrosion in supersaturated water (Fabry et al. 2008). At the moment we can, however, not explain this effect.

Whiteley (2011) suggests that calcification in crustaceans is likely to be less affected by OA for several reasons. These invertebrates produce the more stable form of CaCO_3, calcite (97% in barnacle shells; Barnes et al. 1976; Bourget 1987, Findlay et al. 2010a) rather than the more soluble aragonite. Calcification is realized in compartments isolated from external environment. And, finally, HCO^{3-} is used for CaCO_3 precipitation rather than CO_3^{2-}. This assumption is supported by our data, since net-calcification is positive even under corrosive conditions (leading to shell dissolution) indicative of substantial $CaCO_3$ production. Shell dissolution in barnacles should be hindered by the existence of a protecting epicuticula (Ries et al. 2009; Rodolfo-Metalpa et al. 2011), which covers the outer shell layer (Costlow 1956). When this epicuticula is locally damaged (arrows in Fig. 5), $CaCO_3$ structures become exposed to corrosive waters. Similarly, blue mussels (*Mytilus edulis*) are naturally protected by a protective periostracum, which has been demonstrated to resist various chemicals (Waite 1979). When this periostracum is damaged, mussels experience shell dissolution under corrosive water conditions (Thomsen et al. 2010).

To which extend these corrosions will affect the performance of barnacles and their sensitivity to predation is unexplored. When combined with high-food conditions, as typical for coastal Baltic regions (The BACC author team 2008), OA did not significantly affect breaking resistance. Under predator free conditions, in any case, barnacles thrive under even severely elevated OA, irrespective of shell corrosion. Increased shell strength with increasing food availability was probably based on larger size of barnacles under high-food conditions. Although breaking resistance was corrected by size, a nonlinear increase in shell strength with size can explain these findings. In an earlier study investigating OA effects on a congeneric barnacle species, at pH 7.4, calcification was increased at the lower base, i.e. the active growth region, but shell-strength at the central parts of the shell plates were compromised (McDonald et al. 2009).

As previously described by Pansch et al. (2012), *A. improvisus* larvae are remarkably tolerant to OA. Cyprids utilize energy reserves from the previous larval phase for metamorphosis to the juvenile and early juvenile development until their digestive system is fully developed (Anderson 1994; Thiyagarajan et al. 2003). Settlement of cyprids metamorphosis to juveniles is associated with high mortality (Gosselin and Qian 1997; Jarrett 2003; Thiyagarajan et al. 2005; Shanks 2009; Gosselin and Jones 2010) and considered the most critical step in the life-history of barnacles (Thiyagarajan et al. 2002; Nasrolahi et al. under review in Marine Biology). Nevertheless, in the present study, neither the nauplius larvae nor the metamorphosis to the cypris or to the juvenile barnacle were impacted by even severe OA. As one of only very few studies, McDonald et al. (2009) observed the entire life cycle of an barnacle (*A. amphitrite*) under OA stress but could also not detect any carry-over effects from one to the other life-history stage. In the present study, we furthermore demonstrate that adult life-history does also not affect the performance of F1 larvae and juveniles. This was true for the feeding as well as the acidification treatments on the parents. Also the early growth phase of the F1 juveniles was unaffected by OA as the juvenile phase of the parents (this study and Pansch et al. under review in Marine Biology).

In summary, Kiel Fjord *A. improvisus* seems to tolerate high acidification levels well (see also Pansch et al. 2012; Pansch et al. under review in Marine Biology). Although shell corrosion was observed, none of the fitness aspects examined was negatively impacted by OA, not even reproduction and early F1 performance. In contrast to this observed high tolerance of Kiel Fjord barnacles, however, natural growth rates of the acorn *Balanus glandula* were reduced during upwelling events along the central coast of Oregon, USA (Sanford and Menge 2001; see also Philips 2005; Skinner et al. 2007), possibly explained by reduced OA when upwelling occurred.

Tjärnö barnacles from a more oceanic environment

In contrast to earlier observations on *A. improvisus* (Pansch et al. 2012; Pansch et al. under review in Marine Biology) and to the observations in the present study on the Kiel population, *A. improvisus* from Tjärnö was negatively impacted by OA of nominally 3000 µatm $p\mathrm{CO}_2$. Barnacle growth was unaffected by acidification levels predicted for the open ocean by the end of this century (~1000 µatm $p\mathrm{CO}_2$; Caldeira and Wickett 2005) when enough food was accessible but was reduced under reduced food. DW, AW and CI were only significantly affected by feeding but the general response patterns were the same as for diameter growth. This conditional OA impact, however, vanished under prolonged treatment (>4 weeks). This may be due to the fact that mortality was 10-20 times higher than in the Kiel population (this study and Pansch et al. under review in Marine Biology) and that sensitive individuals may have died by week 5. Under the most severe OA treatments mortality was almost twice as high as under lowest OA. This selective mortality under OA stress and the reduced sensitivity of the survivors may be, however, indicative of a substantial potential for adaptation.

Population specific responses

The fact that $p\mathrm{CO}_2$ treatments differed slightly between the Kiel (477, 966 and 2720 µatm) and the Tjärnö (562, 1123 and 3211 µatm) experiment and the fact that we did not interchange barnacles from one to the other experimental setup does not allow direct comparisons of the two experiments. We show, however, some evidence that barnacles from the Tjärnö Archipelago respond more sensitively to OA than barnacles from the Kiel Fjord. Barnacle mortality was 10-20 times higher in the Tjärnö population compared to the

Kiel population, while mortality in general was low in both experiments compared to studies on other barnacle species (Findlay et al. 2009; Findlay et al. 2010a, b). Barnacles from Kiel showed no growth response to OA even under the most severe $p\mathrm{CO}_2$ treatments (2720 µatm) while Tjärnö barnacles already suffered from 1123 µatm $p\mathrm{CO}_2$ when food was limited. This supports the hypothesis that barnacles from fluctuating habitats are more tolerant to OA than barnacles from more stable oceanic environments. Possibly, the Kiel population is pre-selected to environmental stress.

It was reported that genotype frequencies of acorn barnacles in high and low intertidal populations were similar in freshly settled juveniles but showed strong allelic differences at loci associated with stress tolerance after a period of post-settlement mortality (Schmidt and Rand 2001; Sanford and Kelly 2011), referred to as “phenotype-environment mismatch” (Marshall et al. 2010). Thus it is likely that over many generations, selective mortality on barnacles could have generated a less sensitive barnacle population with respect to OA. Similar results were reported in a comparison of polar and sub-polar barnacle populations of *S. balanoides* (Findlay et al. 2009, 2010a, b, c). A comparison of *Mytilus spp*. from the North- and Baltic Sea indicates, that long-term acclimation to elevated $p\mathrm{CO}_2$ in the Baltic Sea likely increases the ability to calcify under acidified conditions (Michaelidis et al. 2005; Ries et al. 2009; Thomsen et al. 2010). Although invertebrate larvae are generally believed to be more sensitive to environmental stressors (Gosselin and Qian 1997), barnacle larvae from Tjärnö showed a similar insensitivity to OA (Pansch, unpublished Data) as did barnacle larvae from Kiel (Pansch et al. 2012; present study). Larvae from the related barnacle species *A. amphitrite* showed to be also rather insensitive to OA stress (McDonald et al. 2009).

The two habitats differ strongly with respect to the salinity, with a higher mean and stronger fluctuations in the Tjärnö Archipelago. Since *A. improvisus* thrives better in more brackish habitats (Nasrolahi et al. 2012; Nasrolahi et al. under review in Marine Biology), these high salinities might act as an additional stressor to barnacles leading to a higher sensitivity towards OA stress.

From the present study and from earlier investigations it becomes evident that OA driven responses of barnacles are largely depending on the species and on the population investigated (McDonald et al. 2009; Findlay et al. 2009; Findlay et al. 2010a, b, c; Pansch et al. under review in Marine Biology). Supporting a seasonality in the response of barnacles to OA, Pansch et al. (under review in Marine Biology) demonstrates that a cohort of *A. improvisus* individuals collected in autumn suffered from OA (>1930 µatm $p\mathrm{CO}_2$)

after 10 weeks while in the present study with a barnacle cohort collected in early summer, even after 20 weeks no effects of OA (up to 2720 µatm $p\mathrm{CO_2}$) were observed. Thus summer cohorts of barnacles might be more resilient to OA than autumn cohorts.

Conclusions

Seasonal fluctuations in carbonate chemistry of shallow coastal areas can be tremendous and local organisms might have adapted to cope with these fluctuations over many generations. This study supports the hypothesis that barnacle populations from fluctuating $p\mathrm{CO_2}$ environments are more tolerant to OA than barnacles from more stable $p\mathrm{CO_2}$ habitats. In the course of global change, however, atmospheric CO_2 concentrations will continue to rise (IPCC 2007) and will amplify OA in coastal waters (Melzner et al. 2012) and thus will, most likely, impair barnacles' performance in the long term. Eutrophication and thus increasing food availability as well as other factors such as warming have the potential to mitigate the negative effects of OA. A potential for adaptation of these organisms to OA over many generations seems to further exist. Consequently, the predicted OA for the next couple of hundred years does not represent a major direct threat for this species. Community structures are nevertheless susceptible to possible changes in an acidified ocean due to species-specific differences in sensitivities to OA within a community (Fabricius et al. 2011; Hale et al. 2011).

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Tables

Table 1: Feeding treatments in cells per ml of *Chaetoceros calcitrans* and *Skeletonema costatum* (1:1 mixture) and in number of individuals of brine shrimp nauplii (*Artemia salina*) as well as total C (after Troedsson et al. 2005: *Chaetoceros calcitrans* = 4.6 ± 0.3 pg C cell^{-1}; Granum et al. 2002: *Skeletonema costatum* = 19 ± 4 pg C cell^{-1}; Hii et al. 2008: *Artemia salina* nauplii = 0.905 µg C Ind^{-1}) over the different feeding treatments in the Kiel experiment over 20 weeks (equivalent to the Tjärnö experiment).

week	high-food diatoms [cells ml-1]	Artemia [# of individuals]	low-food diatoms [cells ml-1]	Artemia [# of individuals]	high-food C [µg]	low-food
0	64000		13000		2676	544
3	64000	3000	13000	600	5353	1087
4	64000	6000	13000	1200	8106	1630
5		11829		2403	10705	2175
6		20699		4200	18733	3801
7		29570		6000	26761	5430
8		38441		7688	34789	6958
9		44355		8871	40141	8028
10		59140		11828	53522	10704
11		65054		13011	58874	11775
'		'		'	'	'
20		65054		13011	58874	11775

Table 2: In-Situ measurements of temperature, salinity, pH_{NBS}, pH_T and dissolved inorganic carbon (C_T) as well as calculated total alkalinity (A_T), pCO$_2$, $\Omega_{Calcite}$ and $\Omega_{Aragonite}$ of the Kiel- as well as of the Tjärnö experiment under different feeding × acidification treatment combinations as well as of the water at the respective field-sites (Kiel: June - October, Tjärnö: August and September; mean ± s.d.).

		In-situ measurements					calculations			
	air pCO$_2$ [ppm]	T [°C]	Sal	pH NBS	pH Total	C_T [µmol kg^{-1}]	A_T [µmol kg^{-1}]	pCO$_2$ [µatm]	Ω_{Ca}	Ω_{Ar}
Kiel low-food	ambient	19.5 ± 0.4	17.0 ± 1.8	8.06 ± 0.06	8.01 ± 0.08	1819.6 ± 98.1	1934.0 ± 114.6	453.6 ± 91.7	2.69 ± 0.52	1.62 ± 0.32
	1120			7.71 ± 0.05	7.72 ± 0.07	1907.1 ± 100.3	1943.7 ± 114.2	949.7 ± 158.3	1.46 ± 0.30	0.88 ± 0.19
	4000			7.19 ± 0.04	7.29 ± 0.04	2048.0 ± 107.7	1978.0 ± 106.4	2690.4 ± 253.6	0.56 ± 0.07	0.34 ± 0.04
Kiel high-food	ambient			8.12 ± 0.05	7.95 ± 0.08	1738.9 ± 155.9	1833.1 ± 174.6	499.0 ± 103.8	2.27 ± 0.60	1.37 ± 0.37
	1120			7.75 ± 0.04	7.68 ± 0.09	1817.9 ± 162.6	1846.9 ± 178.4	981.5 ± 181.1	1.31 ± 0.42	0.79 ± 0.26
	4000			7.21 ± 0.03	7.26 ± 0.05	1972.6 ± 154.0	1898.6 ± 158.3	2750.0 ± 304.6	0.52 ± 0.11	0.31 ± 0.07
Kiel Fjord		16.7 ± 1.4	16.8 ± 1.9	7.91 ± 0.26	7.86 ± 0.32	1965.4 ± 133.1	2025.1 ± 116.8	780.4 ± 990.2	1.89 ± 0.98	1.12 ± 0.60
Kiel F1 larvae and juveniles	ambient	20.2 ± 0.2	20.4 ± 0.3	8.18 ± 0.05	8.04 ± 0.02	2139.2 ± 64.0	2309.1 ± 69.1	461.2 ± 32.7	3.78 ± 0.27	2.32 ± 0.16
	1120			7.79 ± 0.05	7.77 ± 0.04	2291.8 ± 44.6	2367.7 ± 52.0	957.5 ± 91.7	2.22 ± 0.24	1.36 ± 0.15
	4000			7.25 ± 0.05	7.46 ± 0.07	2477.6 ± 86.6	2461.2 ± 94.6	2148.6 ± 322.1	1.18 ± 0.21	0.73 ± 0.13
Tjärnö low-food	ambient	19.7 ± 0.5	19.3 ± 4.0	8.12 ± 0.09	7.99 ± 0.05	2122.2 ± 301.1	2268.1 ± 347.5	526.4 ± 58.2	3.26 ± 0.89	2.02 ± 0.59
	977			7.79 ± 0.12	7.66 ± 0.04	2087.6 ± 334.2	2122.2 ± 350.6	1141.7 ± 151.00	1.48 ± 0.39	0.91 ± 0.26
	3000			7.32 ± 0.04	7.23 ± 0.03	2265.6 ± 343.3	2179.3 ± 336.7	3255.9 ± 429.7	0.59 ± 0.13	0.36 ± 0.09
Tjärnö high-food	ambient			8.08 ± 0.08	7.93 ± 0.07	2064.8 ± 337.7	2182.8 ± 379.1	596.8 ± 98.6	2.76 ± 0.88	1.70 ± 0.57
	977			7.80 ± 0.17	7.67 ± 0.04	2090.4 ± 380.9	2129.4 ± 395.4	1104.1 ± 193.1	1.54 ± 0.40	0.95 ± 0.26
	3000			7.32 ± 0.05	7.25 ± 0.03	2297.4 ± 326.9	2215.7 ± 319.5	3164.6 ± 421.0	0.62 ± 0.12	0.38 ± 0.08
Tjärnö Archipelago		17.3 ± 1.3	20.9 ± 3.9	8.16 ± 0.10	8.05 ± 0.11	1849.1 ± 274.8	1994.7 ± 339.3	401.5 ± 65.1	3.13 ± 1.35	1.92 ± 0.86

Table 3: Repeated Measures PERMANOVA results of growth in basal diameter, dry weight and ash weight as well as the condition index of *Amphibalanus improvisus* from Kiel Fjord over 20 weeks under different feeding × acidification treatment combinations using Bray Curtis similarity matrices with 9999 permutations (N=8; p (MC) = p value after Monte Carlo correction). Significant effects are in bold.

		d.f.	SS	MS	Pseudo-F	p (perm)	Unique perms	p (MC)
basal diameter	feeding	1	10690.000	10690.000	1219.600	**<0.001**	9929	**<0.001**
	acidification	2	3.439	1.719	0.196	0.955	9948	0.954
	time	3	98052.000	32684.000	3729.000	**<0.001**	9947	**<0.001**
	feeding × acidification	2	4.613	2.307	0.263	0.915	9936	0.913
	feeding × time	3	4727.300	1575.800	179.780	**<0.001**	9963	**<0.001**
	acidification × time	6	15.476	2.579	0.294	0.993	9914	0.993
	feeding × acidification × time	6	9.953	1.659	0.189	1.000	9930	0.999
dry weight	feeding	1	37365.000	37365.000	406.160	**<0.001**	9935	**<0.001**
	acidification	2	83.789	41.894	0.455	0.903	9928	0.898
	time	3	335270.000	111760.000	1214.800	**<0.001**	9939	**<0.001**
	feeding × acidification	2	101.200	50.600	0.550	0.838	9943	0.817
	feeding × time	3	86930.000	28977.000	314.980	**<0.001**	9944	**<0.001**
	acidification × time	6	340.830	56.805	0.617	0.935	9897	0.930
	feeding × acidification × time	6	287.600	47.933	0.521	0.981	9899	0.977
ash weight	feeding	1	34611.000	34611.000	256.180	**<0.001**	9947	**<0.001**
	acidification	2	150.730	75.365	0.558	0.904	9916	0.824
	time	3	336690.000	112230.000	830.670	**<0.001**	9945	**<0.001**
	feeding × acidification	2	242.180	121.090	0.896	0.555	9911	0.507
	feeding × time	3	82905.000	27635.000	204.540	**<0.001**	9924	**<0.001**
	acidification × time	6	481.690	80.282	0.594	0.985	9865	0.953
	feeding × acidification × time	6	674.880	112.480	0.833	0.772	9867	0.697
condition index	feeding	1	11715.000	11715.000	71.205	**<0.001**	9942	**<0.001**
	acidification	2	207.080	103.540	0.629	0.727	9942	0.688
	time	3	12162.000	4053.900	24.640	**<0.001**	9923	**<0.001**
	feeding × acidification	2	207.770	103.890	0.631	0.733	9937	0.687
	feeding × time	3	3292.600	1097.500	6.671	**<0.001**	9922	**<0.001**
	acidification × time	6	1490.200	248.360	1.510	0.075	9916	0.095
	feeding × acidification × time	6	709.590	118.260	0.719	0.818	9907	0.768

Table 4: PERMANOVA and Repeated Measures PERMANOVA results of mortality, moults and larval release of *Amphibalanus improvisus* from Kiel Fjord under different feeding × acidification treatment combinations using Euclidean distance matrices with 9999 permutations (N=8 for mortality; N=5 for moults and larval release; p (MC) = p value after Monte Carlo correction). Significant effects are in bold.

		d.f.	SS	MS	Pseudo-F	p (perm)	Unique perms	p (MC)
mortality	feeding	1	0.008	0.008	1.002	0.335	9809	0.328
	acidification	2	0.034	0.017	2.067	0.133	9955	0.139
	feeding × acidification	2	0.003	0.002	0.202	0.834	9949	0.818
moults	feeding	1	217.270	217.270	0.690	0.413	9831	0.398
	acidification	2	3720.000	1860.000	5.910	**0.003**	9950	**0.004**
	time	3	0.000	0.000	0.000	1.000	9951	1.000
	feeding × acidification	2	248.810	124.400	0.395	0.680	9949	0.678
	feeding × time	3	1045.300	348.440	1.107	0.350	9949	0.357
	acidification × time	6	1761.600	293.600	0.933	0.487	9943	0.477
	feeding × acidification × time	6	1717.700	286.290	0.910	0.498	9944	0.485
larval release	feeding	1	19391.000	19391.000	30.246	**<0.001**	9871	**<0.001**
	acidification	2	1512.300	756.130	1.179	0.313	9955	0.308
	time	3	0.000	0.000	0.000	1.000	9965	1.000
	feeding × acidification	2	1491.500	745.770	1.163	0.318	9951	0.329
	feeding × time	3	420.040	140.010	0.218	0.885	9955	0.882
	acidification × time	6	2922.800	487.140	0.760	0.612	9933	0.608
	feeding × acidification × time	6	1564.800	260.800	0.407	0.877	9958	0.880

Table 5: Factorial ANOVA results of breaking resistance in week 12 and week 20, net-calcification, larval settlement and larval growth of *Amphibalanus improvisus* from Kiel Fjord barnacles as well as mortality from Tjärnö barnacles under different feeding × acidification treatment combinations (N=8 for breaking resistance; N=6 for net-calcification rate; N=6 for larval settlement; N=4 for juvenile growth; N=6 for Tjärnö mortality). Significant effects are in bold.

			d.f.	SS	MS	F	p
Kiel	breaking resistance wk 12	feeding	1	28781.948	28781.948	7.864	**0.008**
		acidification	2	986.879	493.440	0.135	0.874
		feeding × acidification	2	3721.596	1860.798	0.508	0.605
	breaking resistance wk 20	feeding	1	99069.700	99069.700	11.142	**0.002**
		acidification	2	4360.333	2180.166	0.245	0.784
		feeding × acidification	2	11035.514	5517.757	0.621	0.542
	net-calcification rate	feeding	1	0.204	0.204	0.959	0.335
		acidification	2	5.490	2.745	12.904	**<0.001**
		feeding × acidification	2	0.388	0.194	0.912	0.412
	larval settlement	feeding	1	0.002	0.002	0.175	0.679
		acidification	2	0.010	0.005	0.422	0.659
		feeding × acidification	2	0.031	0.016	1.270	0.296
	postlarval growth	feeding	1	0.001	0.001	1.130	0.303
		acidification	2	0.001	0.001	0.669	0.525
		feeding × acidification	2	0.003	0.002	1.769	0.200
Tjärnö	mortality	feeding	1	0.186	0.186	1.157	0.291
		acidification	2	2.727	1.364	8.482	**0.001**
		feeding × acidification	2	0.029	0.014	0.089	0.915

Table 6: Repeated Measures PERMANOVA results of growth in basal diameter, dry weight and ash weight as well as the condition index and factorial ANOVA of mortality of *Amphibalanus improvisus* from the Tjärnö Archipelago over 5 weeks under different feeding × acidification treatment combinations using Bray Curtis similarity matrices with 9999 permutations (N=6; p (MC) = p value after Monte Carlo correction). Significant effects are in bold.

		d.f.	SS	MS	F/Pseudo-F	p/p(perm)	Unique perms	P(MC)
basal diameter	feeding	1	5104.500	5104.500	157.680	**<0.001**	9911	**<0.001**
	acidification	2	457.690	228.840	7.069	**0.001**	9939	**0.002**
	time	2	3562.200	1781.100	55.018	**<0.001**	9954	**<0.001**
	feeding × acidification	2	69.726	34.863	1.077	0.343	9942	0.345
	feeding × time	2	271.840	135.920	4.199	**0.018**	9954	**0.013**
	acidification × time	4	162.390	40.598	1.254	0.293	9936	0.294
	feeding × acidification × time	4	11.247	2.812	0.087	0.994	9957	0.995
dry weight	feeding	1	19093.000	19093.000	43.984	**<0.001**	9943	**<0.001**
	acidification	2	1400.900	700.430	1.614	0.126	9942	0.133
	time	2	102460.000	51230.000	118.020	**<0.001**	9941	**<0.001**
	feeding × acidification	2	517.420	258.710	0.596	0.767	9943	0.742
	feeding × time	2	33464.000	16732.000	38.545	**<0.001**	9943	**<0.001**
	acidification × time	4	1543.500	385.880	0.889	0.572	9914	0.559
	feeding × acidification × time	4	2477.600	619.410	1.427	0.135	9905	0.144
ash weight	feeding	1	18360.000	18360.000	43.025	**<0.001**	9941	**<0.001**
	acidification	2	1417.500	708.770	1.661	0.118	9929	0.130
	time	2	102680.000	51338.000	120.310	**<0.001**	9952	**<0.001**
	feeding × acidification	2	482.290	241.140	0.565	0.790	9935	0.770
	feeding × time	2	31900.000	15950.000	37.378	**<0.001**	9925	**<0.001**
	acidification × time	4	1534.200	383.560	0.899	0.554	9924	0.550
	feeding × acidification × time	4	2399.600	599.910	1.406	0.153	9928	0.152
condition index	feeding	1	3362.700	3362.700	25.296	**<0.001**	9925	**<0.001**
	acidification	2	183.080	91.542	0.689	0.546	9949	0.525
	time	2	1669.600	834.800	6.280	**0.002**	9942	**0.001**
	feeding × acidification	2	6.140	3.070	0.023	0.998	9954	0.998
	feeding × time	2	618.340	309.170	2.326	0.091	9951	0.095
	acidification × time	4	979.100	244.780	1.841	0.115	9942	0.114
	feeding × acidification × time	4	428.810	107.200	0.806	0.546	9949	0.547

Table 7: Summary table of the achieved results illustrating trans-life cycle eutrophication and OA effects on the barnacle *Amphibalanus improvisus* from two distinct habitats with respect to carbonate chemistry fluctuations (positive effects are indicated in light grey, negative effects are indicated in dark grey).

		eutrophication	acidification
Kiel population	growth (diameter)	positive	-
	growth (DW and AW)	positive	-
	CI	positive	-
	survival	-	-
	moulting	-	increased
	reproduction (larval release)	positive	-
	shell strength (breaking resistance)	positive	-
	net-calcification	-	negative
	shell shape	-	negative
	development of F1 larvae	-	-
	growth of F1 juveniles	-	-
Tjärnö population	growth (diameter)	positive	negative
	growth (DW and AW)	positive	-
	CI	positive	-
	survival	-	negative

Figures

Figure 1: Logged water temperature (**a**) and salinity (**b**) as well as measured pH (NBS scale; **c**) and *p*CO_2 (**d**) during the experimental period of 20 weeks (starting 4th of June 2012) in Kiel Fjord and the Tjärnö Archipelago at the field-sites of the respective barnacle field controls in 1.5 m depth (fit type for **a** and **b**: negative exponential weighted LS).

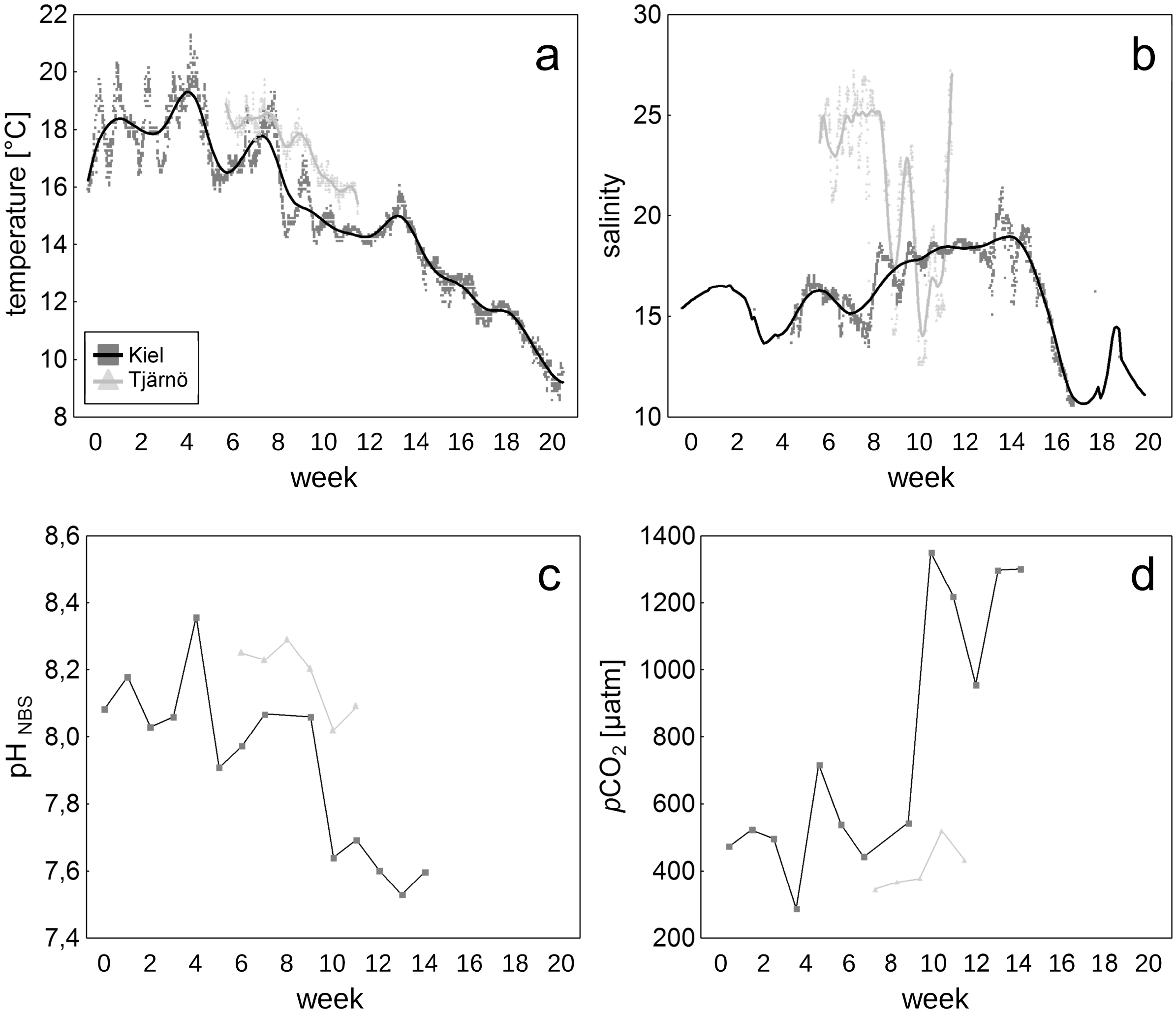


Figure 2: Growth in basal diameter (**a**), dry weight (**b**) and ash weight (**c**; equivalent to shell mass) as well as the condition index (**d**) of *Amphibalanus improvisus* from Kiel Fjord over 20 weeks under different feeding (high-food = +, low-food = -) × acidification treatment combinations and at the field- site (means ± 95% CIs; N=8; n.a. = no values available for the respective treatment combination). Significant effects are indicated by: *=p≤0.05, **=p≤0.01, ***=p≤0.001. Treatment combinations differ according to PERMANOVA pair-wise tests at $p < 0.05$ when they do not share a line connection (feeding treatment comparisons).

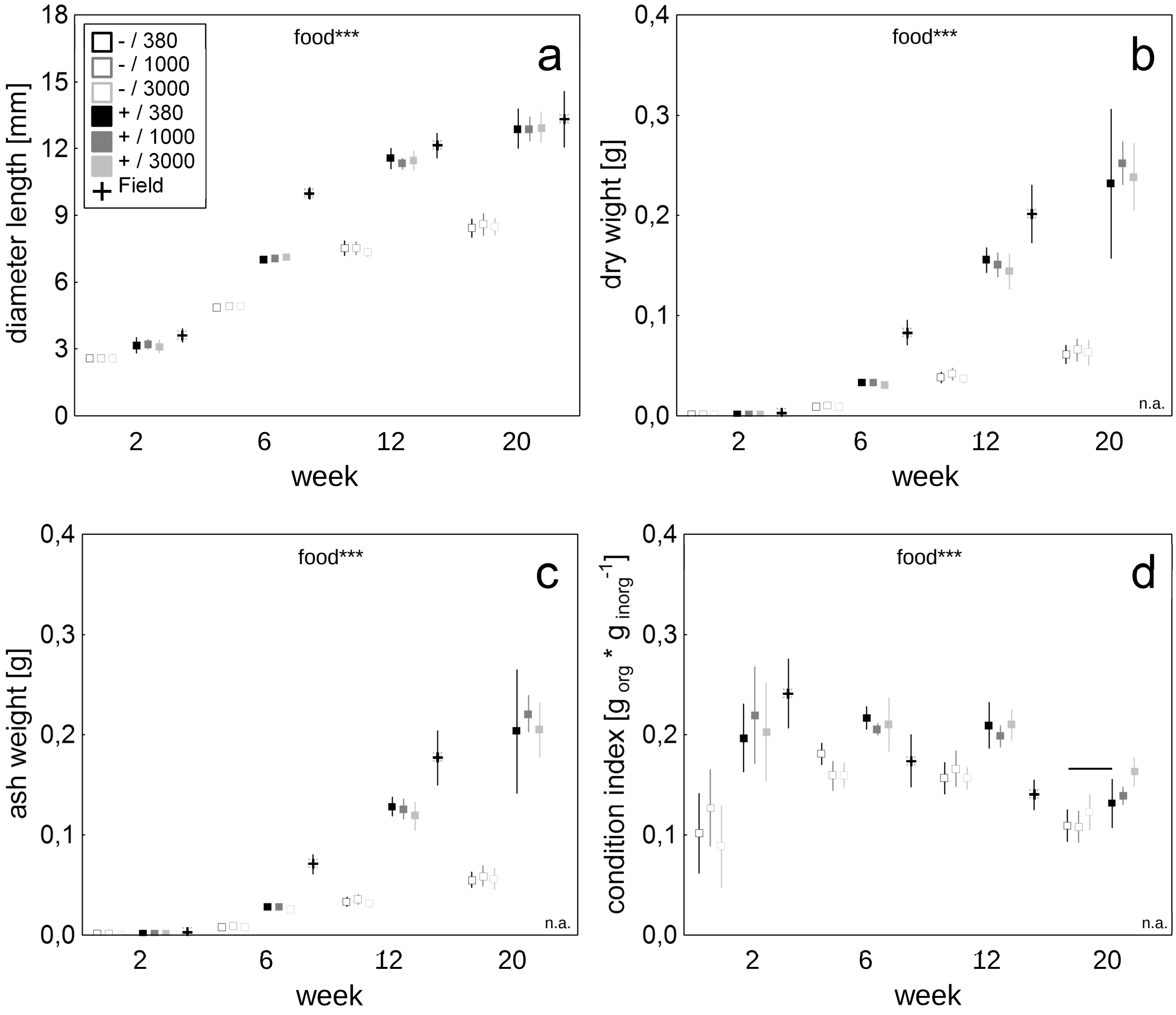


Figure 3: Mortality (**a**), moults (**b**), larval release (**c**) and breaking resistance in week 12 and week 20 (**d**) of *Amphibalanus improvisus* from Kiel Fjord under different feeding (high-food = +, low-food = -) × acidification treatment combinations and at the field-site (means ± 95% CIs; N=8 for a and d; N=5 for b and c; n.a. = no values available for the respective treatment combination). Significant effects are indicated by: $*=p\leq0.05$, $**=p\leq0.01$, $***=p\leq0.001$. Treatment combinations differ according to PERMANOVA pair-wise tests (**b, c**) at $p < 0.05$ when they do not share a higher case letter (acidification treatment comparisons) or when they do not share a line connection (feeding treatment comparisons). Treatment combinations differ according to Fisher's LSD test (**d**) at $p < 0.05$ when they do not share a lower case letter (within week comparisons).

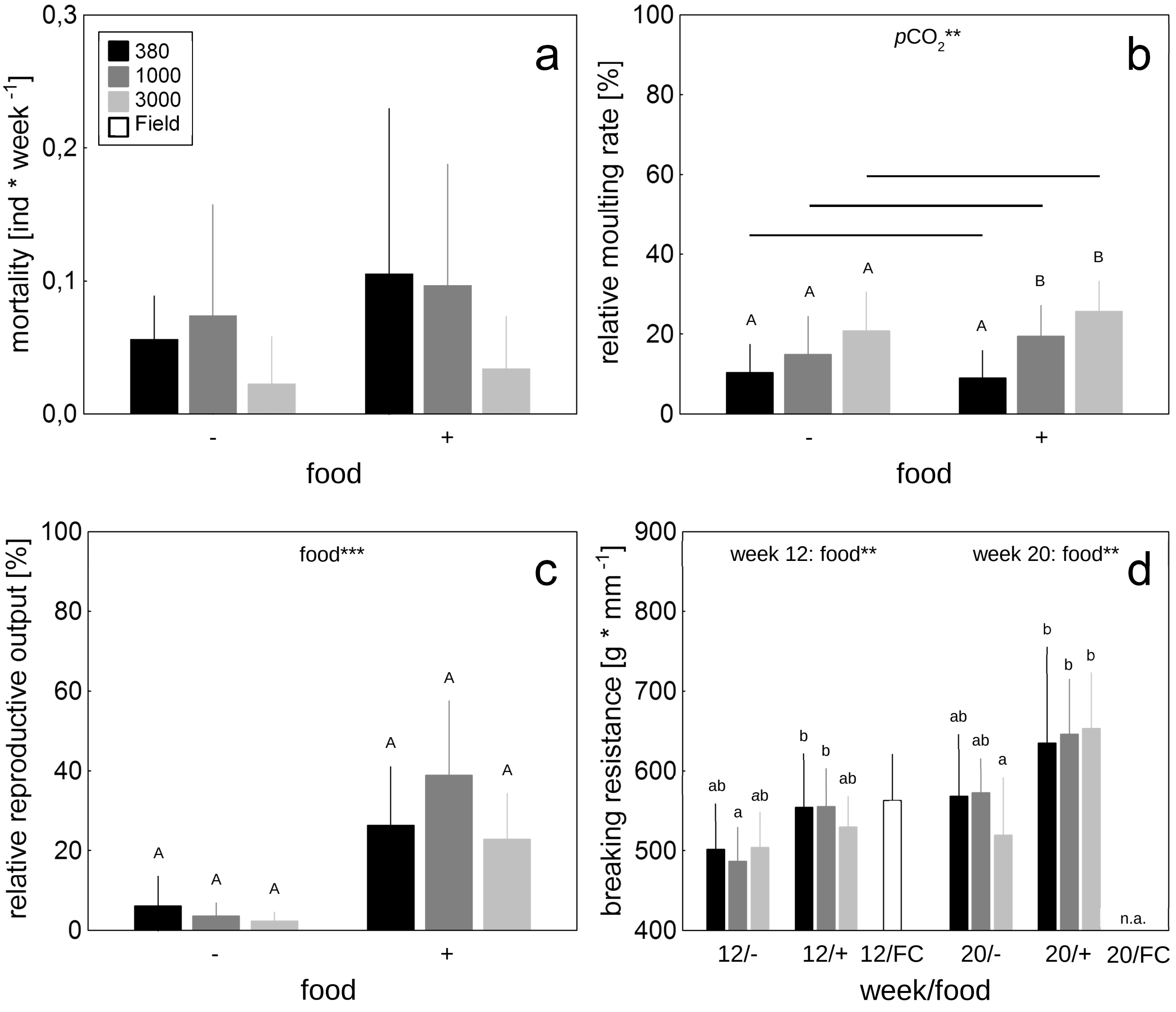


Figure 4: Net-calcification rates of *Amphibalanus improvisus* from Kiel Fjord under different feeding (high-food = +, low-food = -) × acidification treatment combinations (means ± 95% CIs; N=6). Significant effects are indicated by: *=p≤0.05, **=p≤0.01, ***=p≤0.001. Treatment combinations differ according to Fisher's LSD tests at p < 0.05 when they do not share a lower case letter (light-grey area: negative net-calcification).

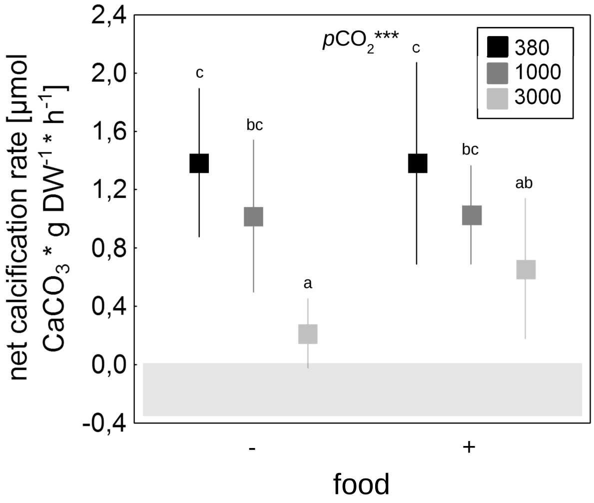


Figure 5: Scanning electron microscopy (SEM) photographs of *Amphibalanus improvisus* from Kiel Fjord under different feeding × acidification treatment combinations in week 19 of the experiment. Grey circles illustrate the point where the force for the breaking resistance measurements was applied. Black arrows display areas of damaged epicuticula (1) and dissolution of the outer calcite layers (2).

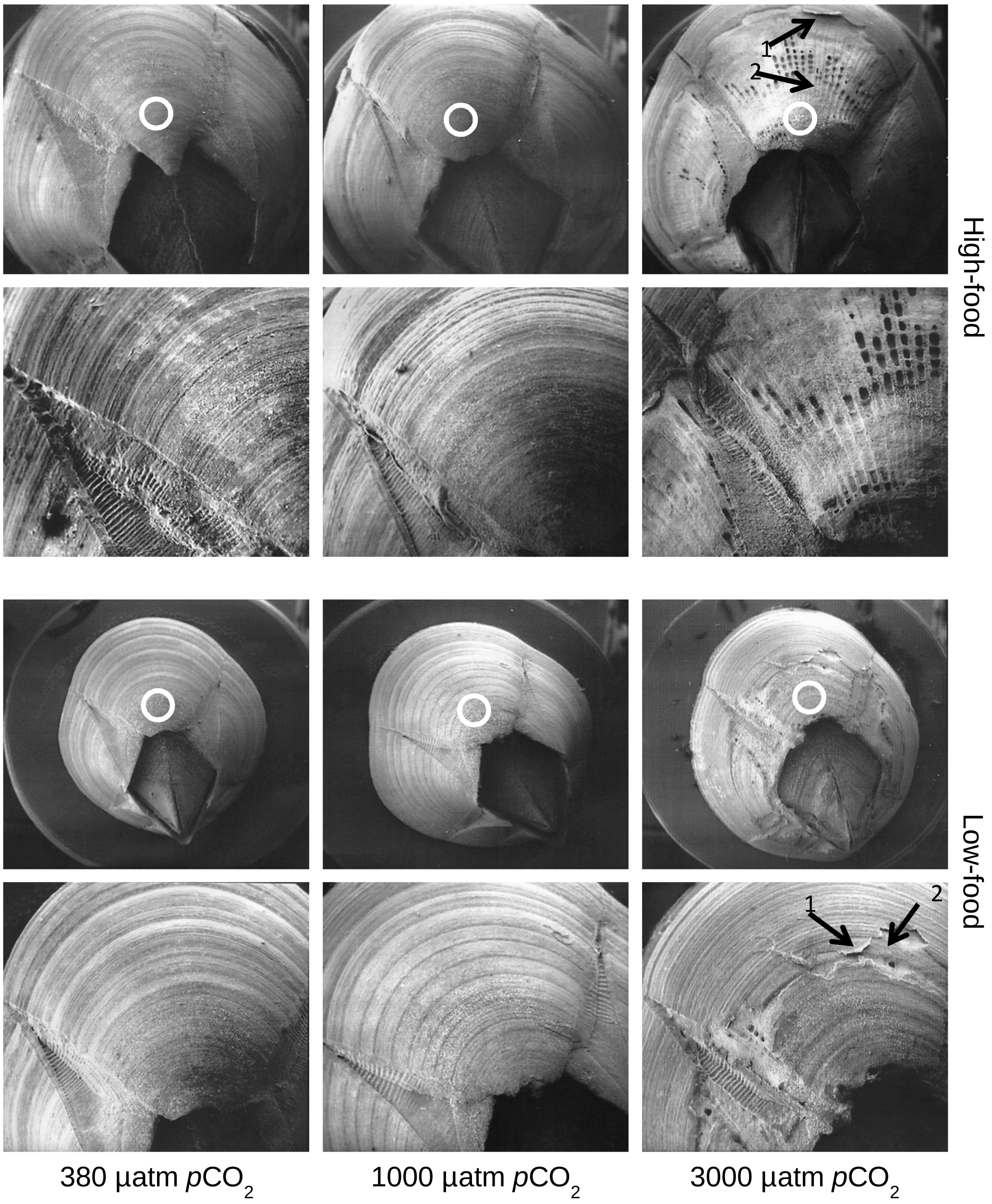


Figure 6: Larval settlement (**a**) and juvenile growth (**b**) of *Amphibalanus improvisus* (Kiel Fjord) F1 individuals under different *p*CO_2 treatments from adults kept under different feeding (high-food = +, low-food = -) × acidification treatment combinations (means ± 95% CIs; N=6 for a; N=4 for b).

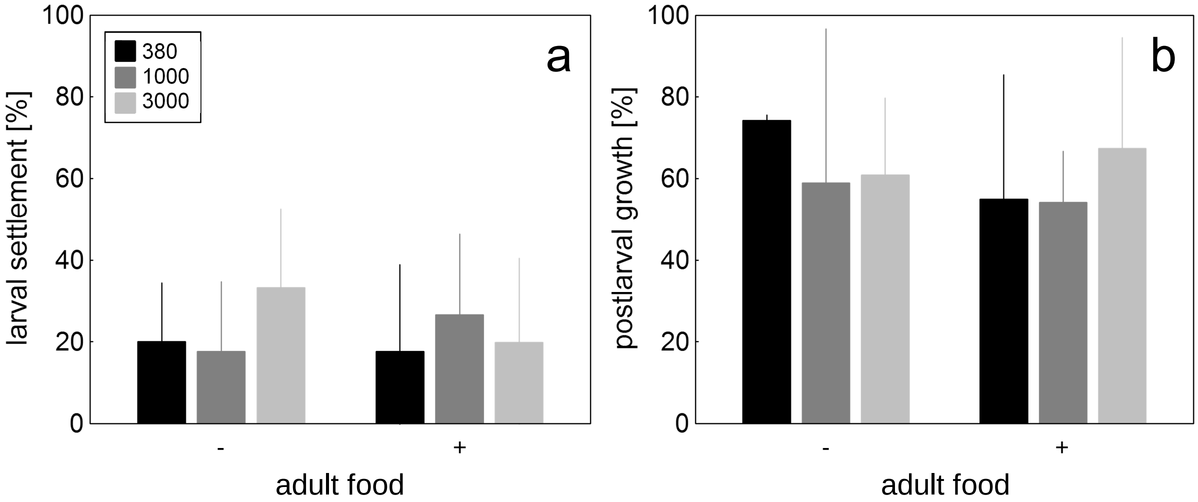


Figure 7: Growth in basal diameter (**a**), dry weight (**b**) and ash weight (**c**; equivalent to shell mass) as well as the condition index (**d**) of *Amphibalanus improvisus* from Tjärnö over 5 weeks under different feeding (high-food = +, low-food = -) × acidification treatment combinations and at the field-site (means ± 95% CIs; N=6; n.a. = no values available for the respective treatment combination). Significant effects are indicated by: *=p≤0.05, **=p≤0.01, ***=p≤0.001. Treatment combinations differ according to PERMANOVA pair-wise tests at p < 0.05 when they do not share a higher case letter (acidification treatment comparisons) or when they do not share a line connection (feeding treatment comparisons).

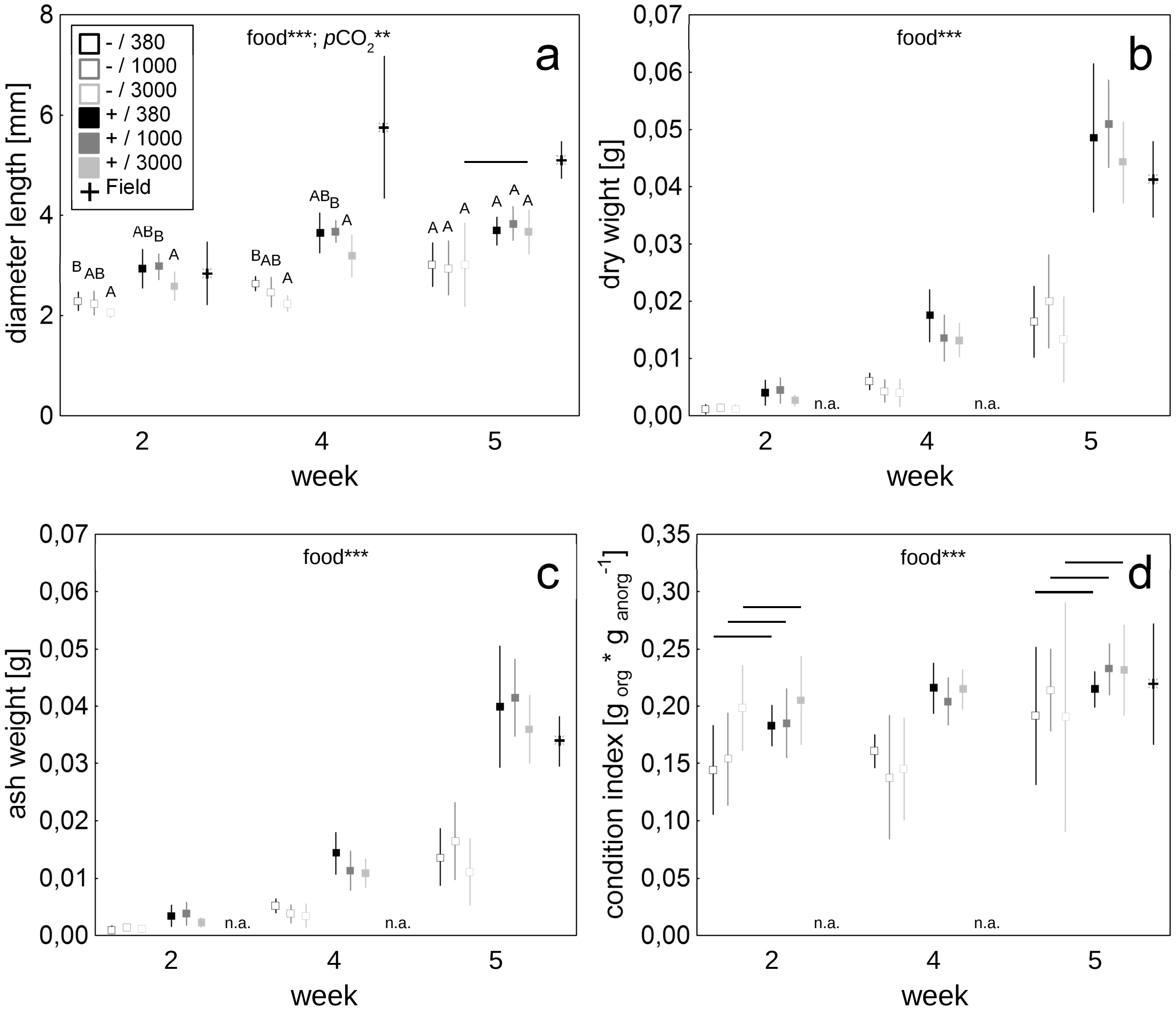
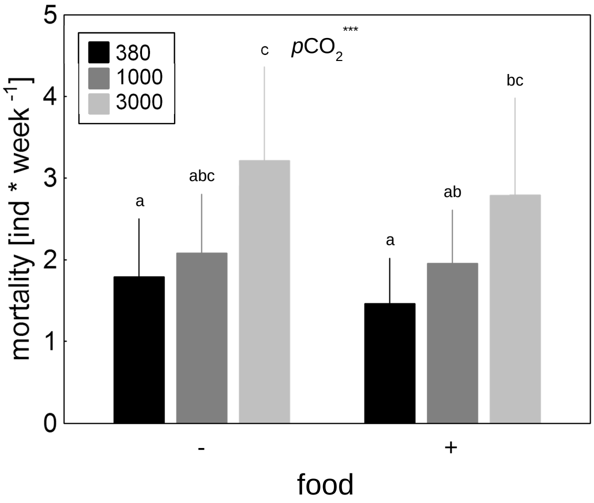


Figure 8: Mortality of *Amphibalanus improvisus* from Tjärnö under different feeding (high-food = +, low-food = -) × acidification treatment combinations (means ± 95% CIs; N=6). Significant effects are indicated by: *=p≤0.05, **=p≤0.01, ***=p≤0.001. Treatment combinations differ according to Fisher's LLSD tests at p < 0.05 when they do not share a lower case letter.



4. General Discussion

The acorn barnacle *A. improvisus* is a dominant marine calcifier within the Western Baltic Sea and can be very abundant in nutrient-rich coastal habitats such as the Kiel Fjord. Where very low salinities disfavour other competitors such as the calcifying mussel *Mytilus edulis*, *A. improvisus* can even entirely dominate the hardbottom environment (Pansch pers. observ.). Calcification in marine organisms is one of the main processes assumed to be impacted OA (Kroeker et al. 2010). OA based changes on the performance of this important calcifier within the species poor Baltic Sea could consequently have major effects on the ecosystem level. Additionally, early life-history stages are assumed to be more sensitive to environmental stressors than adult individuals (Gosselin and Qian 1997). *A. improvisus* provides a complex life cycle, involving various stages such as feeding nauplii and non-feeding cyprids as well as settled juveniles. Thus, this species provides an ideal organism to address a wide range of research questions concerning possible future changes in the marine realm.

In this thesis, I investigated the sensitivity of the barnacle *Amphibalanus improvisus* towards OA stress in combination with additional environmental stressors such as temperature changes and food availability. The study organisms came from the Kiel Fjord, Germany and the Tjärnö Archipelago in Sweden, which allows interpretations at the population level to some extent. As one of the first attempts, this study evaluates the entire life cycle of an invertebrate towards OA in combination with additional stressors.

Temperature treatments used in the present study correspond to mean late summer temperatures when settlement and recruitment of *A. improvisus* peaks within the Kiel Fjord (20°C), down to temperatures occurring in early summer or autumn when the main season of recruitment starts or ends (12 °C) as well as temperatures based on projected warming of surface water within the Baltic Sea (24 or 27 °C; The BACC Author Team 2008). Temperatures of 12 and 20 °C are likely experienced by all life stages of the investigated barnacle species throughout the year already today. Temperatures of 27 °C can be experienced by post-larvae of this species under low water levels when they are exposed to direct sunlight. This temperature does not occur in the main water body within the Kiel Fjord today but will be likely be experienced by Kiel Fjord organisms in the near future (The BACC Author Team 2008). The noticeably high $p\mathrm{CO}_2$ levels for the present study were chosen following the high natural variability in $p\mathrm{CO}_2$ within the Kiel Fjord (Thomsen et al. 2010; Wahl et al. 2010) taking predicted future scenarios into account (Caldeira and

Wicket 2005; Melzner et al. 2012). The feeding treatments applied should represent today's food availability within the Kiel Fjord (high-food) and food limitation for barnacles (low-food). A likely different composition of food between the lab and the fjord environment led to slightly more growth in the field. Nevertheless, strong differences in growth rates of barnacles under the different feeding treatments indicate different levels of energy availability to individuals under high-food and low-food.

Table 4.1: Overview of the sensitivity of *Amphibalanus improvisus* to either moderate (mod) or severe (sev) OA (in combination with other stressors). Barnacles stemmed either from the Kiel Fjord or the Tjärnö Archipelago and juveniles for the experiments were either collected in summer or in autumn. The colours indicate the effect of OA on the different response variables tested: blue = no significant effect; red = negative effect; green: positive effect; grey = not investigated.

			natural		-T		+T				energy availability	
			OA at summer T and food		OA -8°C		OA +7°C		OA +4°C		OA food limited	
			mod	sev	mod	sev	mod	sev	mod	sev	mod	sev
Kiel	nauplii	survival	🟦	🟦	🟦	🟥	🟦	🟦	⬜	⬜	⬜	⬜
		nauplius duration	🟦	🟦	🟦	🟥	🟥	🟦	⬜	⬜	⬜	⬜
	cyprids	survival	🟩	🟩	🟩	🟩	🟩	🟩	⬜	⬜	⬜	⬜
		settlement	🟦	🟦	🟦	🟦	🟦	🟦	⬜	⬜	⬜	⬜
	juveniles (summer)	survival	🟦	🟦	⬜	⬜	⬜	⬜	🟦	🟦	🟦	🟦
		growth	🟦	🟦	⬜	⬜	⬜	⬜	🟦	🟦	🟦	🟦
		CI	🟦	🟦	⬜	⬜	⬜	⬜	🟦	🟦	🟦	🟦
		moulting	🟩	🟩	⬜	⬜	⬜	⬜	⬜	⬜	🟩	🟩
		reproduction	🟦	🟦	⬜	⬜	⬜	⬜	⬜	⬜	🟦	🟦
		shell strength	🟦	🟨	⬜	⬜	⬜	⬜	⬜	⬜	🟦	🟦
		adhesion strength	🟦	🟦	⬜	⬜	⬜	⬜	🟦	🟦	⬜	⬜
		net-calcification	🟥	🟥	⬜	⬜	⬜	⬜	⬜	⬜	🟥	🟥
		shell shape (dissolution)	🟥	🟥	⬜	⬜	⬜	⬜	⬜	⬜	🟥	🟥
		development of F1 larvae	🟦	🟦	⬜	⬜	⬜	⬜	🟦	🟦	🟦	🟦
		growth of F1 juveniles	🟦	🟦	⬜	⬜	⬜	⬜	🟦	🟦	🟦	🟦
	juveniles (autumn)	survival	🟦	🟦	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
		growth	🟦	🟥	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
		CI	🟦	🟦	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
		shell strength	🟦	🟦	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Tjärnö	nauplii	survival	🟦	🟦	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
		nauplius duration	🟦	🟦	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
		size	🟦	🟦	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
	cyprids	size	🟦	🟦	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
	juveniles (summer)	growth	🟦	🟥	⬜	⬜	⬜	⬜	⬜	⬜	🟥	🟥
		CI	🟦	🟦	⬜	⬜	⬜	⬜	⬜	⬜	🟦	🟦
		survival	🟦	🟥	⬜	⬜	⬜	⬜	⬜	⬜	🟥	🟥

Table 1 summarises the sensitivities of *A. improvisus* towards OA (in combination with other stressors) based on data from the present thesis. Nauplius larvae were affected neither by moderate (<1500 µatm) nor by severe (>1500 µatm) OA under summer temperature conditions (20 °C) in the Kiel population (publication I) and under 25 °C in the Tjärnö population (publication II). However, in cooler waters (12 °C) under severe OA as well as in warmer waters 27 °C) under moderate OA the larval development of Kiel individuals was slowed down (publication I). Warming generally increased the survival and rate of development in barnacle larvae, but cypris larvae suffered increased mortality with increasing temperatures (publication I). Cypris size and settlement were unaffected by temperature and OA but survival was enhanced under severe OA (publication I, II). The resilience of juvenile barnacles to OA stress largely depended on the population as well as on the response variable investigated. While survival, growth, CI, reproduction, shell strength as well as development of the F1 generation of Kiel barnacles (summer cohorts) were rather unaffected by OA, moulting frequency increased with increasing acidification of the seawater (publication III and IV). Net-calcification was reduced under increasing acidification with negative impacts on the shell maintenance of adult barnacles, which could be observed as dissolution of the outer shell under severe OA. When a cohort of juvenile barnacles was collected in autumn and investigated under OA scenarios in the laboratory over the winter severe OA negatively affected the growth of these individuals in the long term (publication III). Juvenile barnacles from Tjärnö showed a generally higher sensitivity to OA with reduced growth and survival under moderate and severe OA when food was limited (publication IV). Only severe OA reduced growth and survival when the food availability was increased (publication IV). Food availability in general, had a major and beneficial influence on the performance of juvenile individuals, in both the Kiel and Tjärnö populations of barnacles (publication IV).

4.1. Larval Stages: From Nauplii to Cyprids

Although OA is thought to especially affect early life-history stages (Kurihara 2008; Dupont et al. 2010), nauplius larvae of the barnacle *A. improvisus* were only affected when OA was combined with either decreased or increased seawater temperatures, compared to mean summer temperatures of 20°C when larvae naturally develop and settlement of barnacles peaks. No effects could be detected in 20°C (considered as natural summer conditions) in the Kiel or in 25°C (representing slightly elevated temperatures compared to mean summer temperatures) in the Tjärnö population. This highlights, on the one hand, the importance of investigating multiple environmental factors in ecological experiments, not to overlook simultaneous effects in the light of “global change”, i.e. changes occurring in many environmental parameters in parallel. On the other hand these findings demonstrate that if an overall warming, as expected to occur in the Baltic Sea and other regions in the future (IPCC 2007; The BACC author team 2008), strongly accelerates the larval development, as seen from the present study, net-OA effects can be neglected for the nauplius phase of this species (Fig. 4.1a, b). In comparison, nauplius larvae of copepods (*Acartia erythraea*) showed a significantly increased mortality by about 15 % under $p\mathrm{CO}_2$ values of even 5360 and 10360 µatm (Kurihara et al. 2004) although the nauplii tolerated OA of 2360 µatm $p\mathrm{CO}_2$ (Kurihara et al. 2004). This indicates a certain tolerance to future OA in other crustacean nauplii.

Cypris larvae of *A. improvisus* were not affected by OA. Kiel cyprids rather benefitted from OA but suffered from seawater warming. If an overall warming, as expected to occur in the Baltic Sea in the future (The BACC author team 2008), strongly reduces survival of cyprids, as seen from the present study, the net-OA effects can be neglected, at least for the response variables investigated (Fig. 4.1c, d). Enhanced mortality under elevated temperature was observed likely due to the fact that the non-feeding cypris stage ultimately depends on the amount of energy accumulated during the nauplius stages and elevated temperatures increase metabolic demands and, consequently, the likelihood of energy shortage in cyprids (Thiyagarajan et al. 2002, 2005). It remains, however, unclear why the survival of cypris larvae was favoured under simulated OA. If elevated $p\mathrm{CO}_2$ led to a metabolic depression in cyprids (Pörtner et al. 2004), lowered energy demands could explain the observed higher survival of cyprids under OA. The activity levels of cyprids were, however, not investigated in the present study. The non-feeding cypris' ability to find adequate settlement substrates is important for population dynamics (e.g. Dreano et al. 2006). Thus, any effects weakening their sensory mechanisms, as was for example demonstrated earlier for reef fish (Munday et al. 2009), could have major effects on the recruitment of barnacles. It was, however, not investigated in the present study whether OA affects the sensory mechanisms of cyprids.

The late onset of major calcification in barnacle ontogeny may contribute to the overall small effects of even severe OA on the different life-history stages. While the deposition of significant amounts of calcium carbonate of e.g. echinoderms and bivalves occurs during larval development it is postponed to the phase after settlement in e.g. corals and barnacles (Kurihara 2008). Although early stages of barnacles deposit some calcium carbonate to harden carapace structures (Gohad et al. 2009) these amounts are rather small compared to postlarval calcification (e.g. Findlay et al. 2010a; Nasrolahi 2012). In a recent study, McDonald et al. (2009) observed no OA effect on the growth of *Amphibalanus amphitrite* larvae. Furthermore, nauplii and copepodites of for example *A. tsuensis* and *A. erythraea* were also unaffected by severe OA (e.g. Kurihara et al. 2004; Kurihara and Ishimatsu 2008) while certain calcifying echinoderm larvae seem to be very vulnerable to

OA stress when exposed to simulated OA (e.g. Dupont et al. 2008). Similarly, the early embryogenesis of mussels (*Mytilus galloprovincialis*) was unaffected by exposure to pCO_2 levels of about 2000 µatm, and effects only appeared at the later trochophore stage in which the shell begins to form (Kurihara et al. 2009).

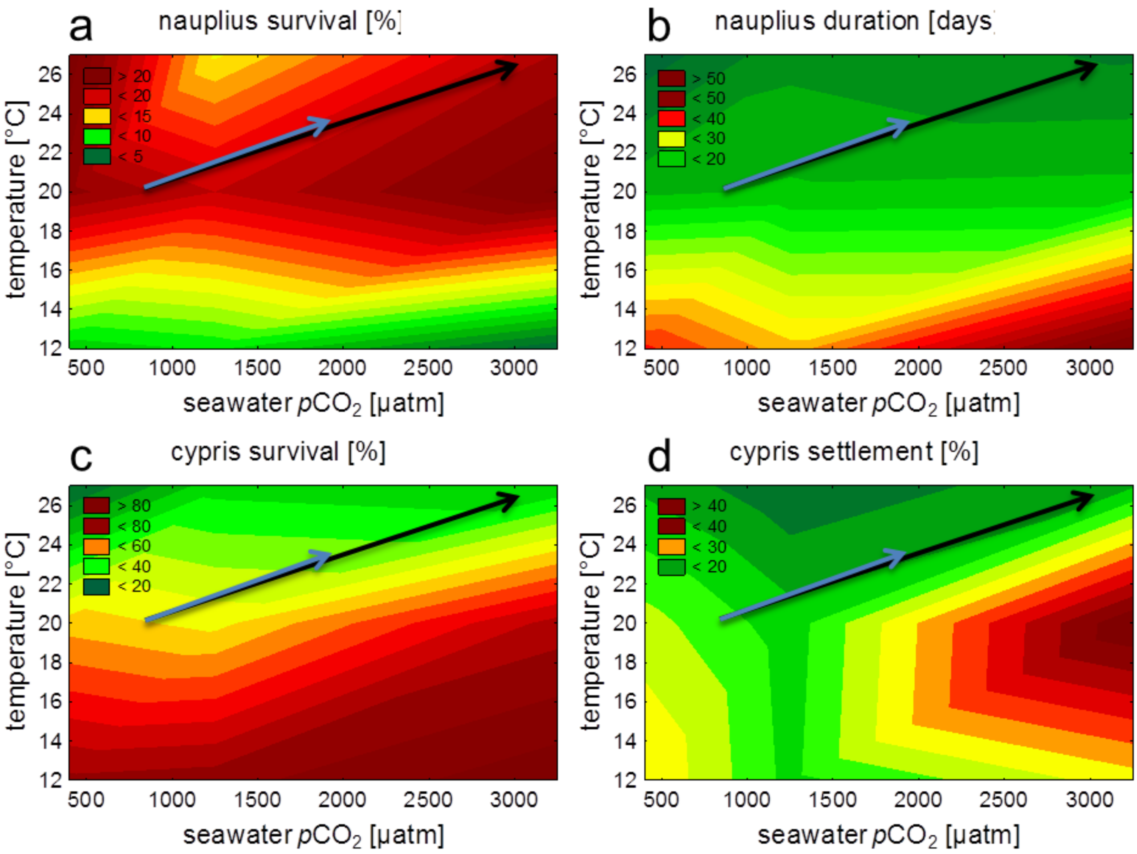


Figure 4.1: Expected responses of *A. improvisus* larvae to future global changes. Wafer-plots represent the nauplius survival (**a**), the nauplius duration (**b**), the cypris survival (**c**) and the cypris settlement (**d**) over a range of temperature and pCO_2 values. Blue arrows indicate future ocean warming of +3 °C and a future seawater pCO_2 of 2000 µatm; black arrows indicate future ocean warming of +6 °C and a future seawater pCO_2 of 3000 µatm (projections after Caldeira and Wicket 2005; The BACC Author Team 2008; Melzner et al. accepted in Marine Biology).

Temperature seems to exert a major control on the larval phases of *A. improvisus* with opposite effects on nauplii and cyprids. To date, no data are available on the interaction between OA and further stressors such as salinity shifts or food limitation regarding their impact on the larval phase of barnacles. I can at this point only speculate about possible effects. Salinity in general, has large but variable effects on the performance of *A. improvisus* larvae. For the Kiel population, Nasrolahi et al. (2012) showed that low salinities of 5 favoured the survival of nauplii while the settlement of cyprids was highest in medium salinities of 15. Settlement of naive cyprids (where nauplii were raised at the ambient conditions of 20°C and a salinity of 15) was highest at salinities of 30. Furthermore, barnacles from Tjärnö performed best at salinities of 15 (Nasrolahi et al. 2012). It seems that the cyprids generally prefer a slightly higher salinity than the nauplii. An expected desalination of seawater within the Baltic Sea will however likely further promote *A. improvisus* larval development in the future.

4.2. From Juveniles to adults and the F1 generation

Juveniles of the barnacle *A. improvisus* from Kiel responded weakly to OA. Mortality remained below 5 % in all experiments and was not affected by OA. The only significant negative effect found on growth was a decreased basal diameter by 5 to 9% under pCO$_2$ values of >1930 µatm after 12 weeks of treatment. This effect was only observed in a cohort collected in late autumn and investigated over winter. This apparent seasonality in barnacles' response to OA is not new and a seasonal pattern has been previously observed for *Semibalanus balanoides* (Findlay et al. 2009, 2010a, b, c). It was postulated that the larval condition can determine the success of juvenile barnacles (Jarrett 2003) and if the "autumn cohort" comprised of larvae with lower fitness, this could likely explain the findings in the present study. It remains, however, unclear why this pattern was not observed in the phase of early juvenile growth but only after 10 weeks. All in all, it remains to be answered whether summer or autumn cohorts contribute to comparable amounts to the overall recruitment of barnacles in the study region or whether the summer cohort has a higher contribution. Thus, interpretations from these findings are difficult.

Warming and increasing food availability had much stronger effects than OA and both of these factors increased growth rates of barnacles. Although warming by +4 °C only briefly affected the growth of barnacles, it decreased the condition index (CI) and increased the breaking resistance, i.e. led to an increased investment into shell production compared to body growth (see also Nasrolahi 2012). Thus, future warming could lead to barnacles being less sensitive to shell-breaking predators (Urban 2007). Although food quality and quantity play an important role for *A. improvisus* larval development (Nasrolahi et al. 2007) no one has investigated the combined effects of food and OA in barnacles so far. Increasing food availability strongly increased the growth of juvenile barnacles as well as the CI and the breaking resistance. Although barnacles invested more energy into soft-tissue (body) compared to shell growth under increased food availability, the shell strength was nevertheless increased compared to the low-food treatments. This effect might, however, result from the strongly increased size under high-food and the fact that size and shell strength are not necessarily linearly correlated. Differences in the results obtained for different barnacles species, and from different experiments, (e.g. Findlay et al. 2009, 2010a, b; McDonald et al. 2009; present study) might originate from species-specific differences but might also have been caused by different feeding conditions in the various experiments. Findlay et al. used concentrations of 1.5×10^4 diatom/flagellate cells ml^{-1} and fed every second day for *S. balanoides* and *E. modestus* (Findlay et al. 2010a) while McDonald et al. (2×10^6 diatom cells ml^{-1} fed daily for *A. amphitrite*; McDonald et al. 2009) and the present study (2×10^5 diatom cells ml^{-1} fed daily for *A. improvisus*) used higher concentrations of food for rearing early post-larvae.

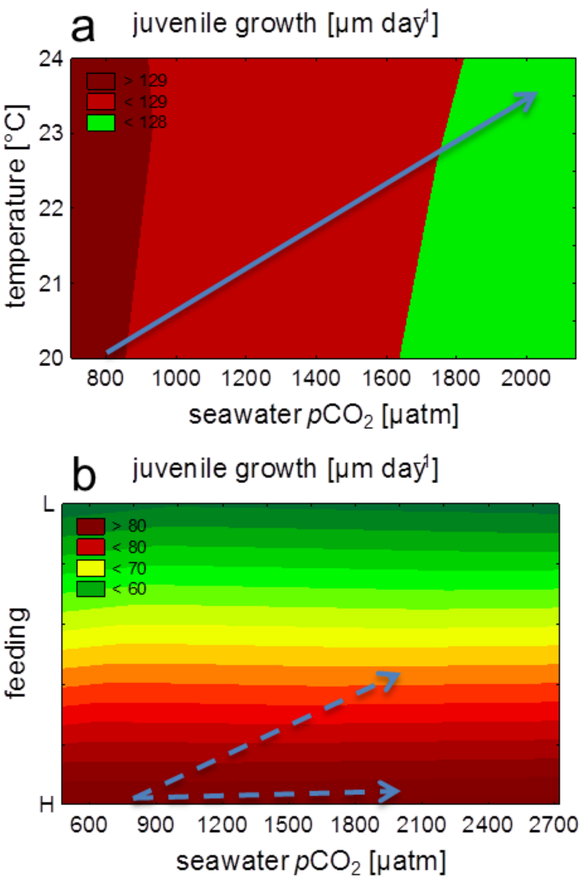


Figure 4.2: Expected responses of juvenile *A. improvisus* to future global changes. Wafer-plots represent the growth estimated over a range of temperature and pCO$_2$ values (**a**) and over a range of food availability (H=high-food, L=low-food, see publication IV for details) and pCO$_2$ values (**b**). Blue arrows indicate future ocean warming of +3 °C and a future seawater pCO$_2$ of 2000 µatm. Dashed lines indicate either stagnation of the eutrophication status or slight improvements in the future (projections after Caldeira and Wicket 2005; The BACC Author Team 2008; Melzner et al. accepted in Marine Biology).

Under a scenario including future warming (IPCC 2007) but assuming stagnation of the actual eutrophication status of seawater (i.e. food availability; The BACC author team 2008), barnacle growth will likely not be significantly impaired by future changes (Fig. 4.2). If I include the expected desalination into the scenario, survival and growth of post-larvae will, however, decrease in the future (Nasrolahi 2012).

Barnacles in general have been reported to moult every 2-3 days (Costlow and Bookhout 1957) and moulting increased almost linearly with increasing OA in the present study. This has previously shown in other crustaceous species, e.g. the shrimp *Palaemon pacificus*, under a pCO$_2$ of 1000 µatm (Kurihara et al. 2008). In our study, moulting frequency did not relate with growth or the CI and for the moment I cannot explain this response pattern. Moulting in crustaceans is essential for growth of the body and increased moulting under OA might not necessarily be a negative response. The importance of these findings need to be, however, considered in future investigations.

The reproduction of barnacles did not depend on the level of OA. This is also in line with studies on copepods (Mayor et al. 2007; Kurihara 2008). In the barnacle *Semibalanus balanoides*, however, OA delayed the embryonic development (Findlay et al. 2009). It is well known that barnacles can regulate their reproductive output depending on the food availability (Hines 1978). This is supported by the present study although I cannot conclude whether food availability impacted reproduction indirectly via higher growth rates of barnacles or directly via energy supply to the gonads.

Figure 4.3: *Amphibalanus improvisus* treated under OA in a $p\mathrm{CO}_2$ of 2720 µatm for 20 weeks.

Most of the investigated parameters in the present study were not significantly impaired by OA although the shells of barnacles were severely dissolving from the outside under the most severe $p\mathrm{CO}_2$ treatment. This became evident from inspections under the binocular (Fig. 4.3) as well as from SEM pictures and net-calcification rate measurements, indicating that, although shell growth can be maintained (see discussion above), the outer exposed areas of the shells suffer from CaCO_3 undersaturation. It could be that adult barnacles reallocated energy into reproduction rather than into shell maintenance and that shell characteristics are not as important to the barnacles in this later state of life. Reduced net-calcification in under-saturated waters is a common phenomenon among diverse invertebrate species (e.g. Ries et al. 2009). Since, severe shell dissolution did not impair the reproductive output and the larval conditions of *A. improvisus* and possibly did not lethally impact other earlier investigated organisms (e.g. Ries et al. 2009), it remains unclear to which extent dissolution of outer shell material impairs the organism itself.

Many calcifying organisms (e.g. scleractinian corals, coralline algae, foraminifera and also crabs) are believed to raise the pH at the site of calcification to facilitate the precipitation of CaCO_3 (Ries et al. 2009 and references therein). OA will reduce the CO_3^{2-}

concentration of seawater and likely inhibit calcification and intensify dissolution of existing shell (Langdon et al. 2000; Langdon and Atkinson 2005; Kleypas et al. 2006). Organisms, such as crustaceans, which are capable of regulating the pH at the site of $CaCO_3$ precipitation, might thus be less sensitive to future OA (Whiteley 2011). Since pH regulation or the transports of ions are processes believed to be costly, this should however, result in either reduced reproduction or performance of the recruits. Nevertheless, none of these processes was significantly affected by OA in the present study. Food availability, in contrast, impacted the reproductive output (see also Hines 1978) but had no impact on the larval conditions (i.e. the performance of the F1 larval generation and the post-larval performance).

Many earlier investigations have demonstrated that carry over effects significantly impair subsequent life-history stages in barnacles (Thiyagarajan et al. 2002, 2003, 2007). The settlement of the cypris larva as well as the metamorphosis and the early post-larval stage has been suggested to be the bottleneck in the development of *A. improvisus* (Nasrolahi 2012). Investigating the effects of the adults' life history under OA on the overall development of nauplius and cypris larvae towards the point of settlement, metamorphosis and post-larva did not confirm these hypotheses, at least not under OA stress. The development of the nauplii and cyprids under OA did not affect the subsequent juvenile growth of *A. improvisus* as has been suggested earlier for other stressors such as salinity (Thiyagarajan et al. 2002, 2003, 2007). In conclusion, this species has been demonstrated to even withstand the developmental steps under OA well, which have previously been highlighted as the bottleneck in the ontogeny of this species, (Nasrolahi 2012).

In general, temperature and food affected juvenile barnacles much stronger than OA and in the future juveniles from Kiel will likely not suffer from OA as is expected from oceanic scenarios. Barnacles from Tjärnö showed, however, a higher sensitivity to OA with reduced growth and reduced survival under moderate and severe OA when combined with food limitation and under severe OA when food availability was increased. This study indicates that not only different taxa or species of invertebrates respond differently to OA (e.g. Kroeker et al. 2010) but also different populations of the same species can react contrarily (discussed in Dupont et al. 2010).

4.3. Variability of the carbonate chemistry within Kiel Fjord and potential for adaptation in barnacles

In open ocean time series (e.g. of the Atlantic Ocean) the rate of seawater pCO_2 correlates nicely with atmospheric pCO_2 increases (Takahashi et al. 2009) leading to rather linear shifts in seawater pH over time (e.g. Doney et al. 2009; Feely et al. 2009; Ishii et al. 2011). In Polar regions, pH decreases even faster than observed in oceanic time series such as for example the Hawaii Ocean Time Series (e.g. Olafsson et al. 2009). In contrast to the open ocean in general coastal habitats are characterized by much stronger variability of the carbonate chemistry (e.g. Borges and Frankignoulle 1999; Borges et al. 2006; Blackford and Gilbert 2007; Shim et al. 2007; Salisbury et al. 2008; Wootton et al. 2008; Miller et al. 2009; Provoost et al. 2010). The Kiel Fjord has been recently described as a habitat in which the carbonate chemistry varies substantially over the year (Thomsen 2012) with pH/pCO_2 values diverging strongly from open ocean measurements and projections (e.g. Thomsen 2012; present study). But not only shallow coastal bays and enclosed seas show naturally occurring acidification events or substantial variability of the carbonate chemistry. Even entire continental upwelling regions such as the US west coast are characterized by the upwelling of deep-water masses with high pCO_2 loads (Feely et al. 2008; Feely et al. 2010). This water is upwelled upon the continental shelf likely affecting the local flora and fauna in shallow habitats over long periods of time.

In the Kiel Fjord, the upwelling of high- pCO_2 water is caused by different processes. Wind-driven upwelling events transport deep-water masses to the surface (Myrberg and Andrejev 2003; Thomsen 2012). The strong stratification within the Baltic Sea (HELCOM 2003) is the main reason of accumulations of high-pCO_2 water due to the heterotrophic degradation of sedimented plankton blooms (Hansen et al. 1999; Broecker 2003). In the Kiel Fjord and likely in other regions within the Baltic Sea as well as enclosed seas worldwide, eutrophication increased dramatically during the last century (Diaz and Rosenberg 2008; The BACC author team 2008) resulting in seasonal oxygen depletion in deeper waters (Babenerd 1991; Conley et al. 2007; HELCOM 2009). O_2 depletion and CO_2 production are strongly coupled, especially when the exchange with the atmosphere is prevented by stratification, and an enhanced community respiration rises with increasing eutrophication (Cai et al. 2011; Melzner et al. 2012). Following the Boknis Eck time series, the bottom water pCO_2 increased from about 750 μatm in 1957 to today's levels of about 2500 μatm (Thomsen 2012). Thus, an increased eutrophication since the mid of the last century (The BACC author team 2008) likely augmented the magnitude of natural acidification as well as its variability in shallow coastal habitats along the Baltic coasts.

Eutrophication drives anoxia, which can cause local OA. Organisms inhabiting coastal regions have therefore likely experienced an increase in absolute pCO_2 values (decrease in absolute pH values) and also an increase in the variability of the carbonate chemistry for over half a century. This happened, however, in parallel with an increase in food availability for filter feeding invertebrates (The BACC author team 2008; see also Thomsen 2012). Calcification can be a major process affected by “natural acidification” in for example the Kiel Fjord, in which aragonite saturation states are under-saturated during 65% of a year (Thomsen 2012). The correlation between the acidification level and the occurrence of calcifying organisms in the vicinity of natural CO_2 vents has been used to demonstrate the high vulnerability of calcifiers to future OA (e.g. Hall-Spencer et al. 2008). Compared to these relatively small areas of “natural acidification” in the range of several hundreds of meters (Hall-Spencer et al. 2008), large areas such as the Kiel Fjord (Thomsen 2012) or the Eckernförde Bay (Vincent Saderne pers. commun.), as well as even larger regions within the Baltic Sea (Frommel et al. 2012) which temporarily experience undersaturation with respect to $CaCO_{3,}$ could favour genotypes, which are more resistant to OA. While gene flow from adjacent non-impacted areas can reduce the effect of strong natural selection in local populations (Hall-Spencer et al. 2008), local adaptations may arise in larger naturally acidified regions such as the Baltic Sea.

In conclusion, the Kiel Fjord is characterised by a strong seasonal variability of the pCO_2, which might be responsible for local populations of barnacle being more resistant to future OA than populations from other habitats such as the Tjärnö Archipelago (see also Fabricius et al. 2011). Nevertheless, the low alkalinity in the Baltic Sea will lead to stronger pH decreases from to the dissolution of anthropogenic CO_2 into the seawater compared to fully marine environments. From this and the previously discussed characteristics of small-scale coastal habitats, future changes will more severely shift the carbonate chemistry of these coastal shallow habitats (Melzner et al. 2012). Although organisms in coastal habitats are likely adapted to withstand acidification since it already naturally occurs today, the large expected changes due to climate change could still lead to negative impacts on the barnacle *A. improvisus* and on other invertebrates.

Furthermore, small-scale fluctuations in pCO_2 or pH are highly relevant for benthic organisms. Short-term fluctuations experienced by organisms can be remarkable in for example *Fucus* spp. macroalgal meadows with $p\mathrm{CO_2}$/pH values shifting by about 2000 µatm or 1.2 pH units over 24 hours (Vincent Saderne pers. commun.). In the boundary layer, i.e. less than one millimetre above the surface of an algal thallus, O_2 and pH (and likely $p\mathrm{CO_2}$) can fluctuate by orders of magnitude between day and night, i.e. algal photosynthesis and respiration (e.g. De Beer and Larkum 2001; Beer et al. 2008; Spilling et al. 2010; Mathias Fisher, pers. commun.). These fluctuations might also impact the performance of the associated organisms (Woods and Podolsky 2007). Various animals such as bryozoans, tube building polychaetes (e.g. Saderne and Wahl 2012) or barnacles (pers. observ.) commonly settle on living surfaces such as algal thalli. Since the settlement and earliest calcification in barnacles or other benthic calcifiers occurs within the surface's boundary layer, these organisms might be naturally adapted to cope with severe shifts in carbonate chemistry. These organisms could thus be pre-adapted to cope with predicted future changes in the carbonate chemistry.

4.4. Revisiting the Hypotheses

H1 - OA will affect the barnacle A. improvisus: Individuals the barnacle *A. improvisus* from Kiel, in the lab experiments as well in the field, showed a remarkable tolerance to OA. CO_2 Levels predicted for the open ocean by the end of this century (~1000 µatm $p\mathrm{CO_2}$) as well as by 2300 (~2000 µatm pCO2; Caldeira and Wickett 2005; Orr et al. 2005), which are also temporarily encountered by barnacles within their natural habitats already today (Thomsen et al. 2010; Pansch et al. 2012) were tolerated by the investigated individuals under common summer temperature conditions at all life stages. Larval stages from Tjärnö were not affected by OA while juvenile barnacles were negatively impacted by OA. Nevertheless, OA levels predicted for the open ocean by the end of this century (~1000 µatm $p\mathrm{CO_2}$; Caldeira and Wickett 2005; Orr et al. 2005) at present food availabilities were tolerated well by all individuals investigated, including all populations and life stages. When combined with warming scenarios, as well as possible adaptation capabilities of this species, which become evident from comparisons of populations from Kiel and Tjärnö, the barnacle *A. improvisus* will likely not be dramatically impaired by future OA as predicted from oceanic models. Coastal habitats will, however, experience more severe shifts in carbonate chemistry due to anthropogenic CO_2 release than oceanic habitats in the future and seawater $p\mathrm{CO_2}$ values of even 4000 µatm can be expected in the Western Baltic Sea (Melzner et al. 2012). Possible long term effects of this coastal specific OA on this species should, thus not be neglected.

H2 - Early life-history stages of the barnacle A. improvisus are more vulnerable to OA than adult stages: It is widely assumed that early life-history stages would react more sensitively to environmental stressors than their adult stages and causes for mortality at these stages are thought to be important determinants of population dynamics and driving forces in the evolution of the species (Gosselin and Qian 1997). Biotic factors such as predation, competition, energy depletion and disease and abiotic factors such as desiccation, temperature, salinity, water motion and solar radiation have been suggested to be responsible for the observed high mortality in early life stages (Gosselin and Qian 1997 and references therein). Nevertheless, in contrast to previous assumptions, early juvenile mortality might be even higher than the mortality of larval stages (Gosselin and Qian 1997; see also Nasrolahi 2012). Larval phases have also been postulated to be one of the key life-history stages affected by OA (Kurihara 2008). This has been verified for example in brittlestars where the early larval phase suffered from severe mortality under simulated OA (Dupont et al. 2008). Excluding biotic factors such as predation and energy depletion, I observed the highest mortality of *A. improvisus* during the development of nauplius larvae (80-90%). Mortality of cypris larvae was high with 20-80% while juvenile to adult barnacles displayed a very low mortality of less than 5%. Nevertheless, OA affected survival and other fitness related parameters only in very few cases. Nauplius larvae were impaired by OA only when this stress occurred together with temperatures shifts while cypris larvae showed even increased survival under OA. It remains to be investigated, how shell dissolution affects the performance of barnacle post-larvae, although at least for the parameters investigated (growth, CI, Moulting, reproduction), I could not detect any strong negative impacts of OA for the Kiel population. In conclusion, the data on the entire life cycle of the barnacle *A. improvisus* confirm the hypotheses that early life-history stages, in general, suffer a higher mortality than adult stages. I can, however, not confirm the hypothesis that early life-history stages are more vulnerable to OA stress than adult stages. In the Tjärnö population, early post-larval stages were more sensitive to OA than larval stages. The very early post-larval metamorphosis and development to an age of about 48 h post settlement of *A. improvisus* has been discussed to be the bottleneck in the development of this species (Nasrolahi 2012) but only indirect data of this phase of development in response to OA have been obtained herein. This could, however, be addressed in potential future investigations.

H3 - Predicted warming will enhance OA effects on the barnacle A. improvisus: Warming and OA can act in synergy (O'Donnell et al. 2009; Pörtner 2008; Pörtner and Farrell 2008; Parker et al. 2010; Pörtner 2010) and OA can narrow the thermal tolerance window of organisms (Walther et al. 2010). A predicted increase in seawater temperature (IPCC 2007; Meier 2006) will however, in general, accelerate nauplius development of *A. improvisus* (present study; Nasrolahi et al. 2012) and, thus, has the potential to buffer OA effects. Such results were also obtained in sea urchin larvae (*Tripneustes gratilla*) and oysters (*Crassostrea virginica*), where higher temperatures mitigated negative effects of OA (Brennand et al. 2010; Waldbusser et al. 2011). Positive effects of OA on the survival of cypris larvae of *A. improvisus* could further mitigate the negative impact of warming on the survival. Thus, I cannot confirm that temperature, at least in the range investigated, will have additive negative effects on the performance of the barnacle *A. improvisus*.

H4 - Food availability will affect the capacity of A. improvisus to cope with future OA: There are only few data available on the combined effect of OA and food quantity or quality on marine organisms (but see e.g. Thomsen 2012). It can, however, be assumed that food availability would help the organism to maintain increased metabolic rates necessary for energy demanding processes such as active ion regulation, which increases during acidified conditions (Melzner et al. 2009; Whiteley 2011) or increased calcification, which is necessary to overcome the dissolution of shell material under lowered $CaCO_3$ saturation states to maintain shell stability (McDonald et al. 2009, Melzner et al. 2011). Food availability, in general, was a major factor driving the performance of juvenile (e.g. growth) and adult (e.g. reproduction) barnacle individuals of *A. improvisus*. There were however, no statistically significant interactive effects of food availability on the sensitivity of barnacles to OA. Nevertheless, it can be concluded from the experiments on juvenile barnacles from Tjärnö, that barnacle populations which are in general impaired by OA, might overcome moderate OA when the food availability is high but would suffer from even moderate OA under limited food conditions. From field studies, large differences in species responses to OA have been observed between eutrophic and oligotrophic coastal habitats (e.g. Hall-Spencer et al. 2008; Thomsen et al. 2010). While in the highly eutrophic inner Kiel Fjord calcifying organisms largely dominate benthic communities (Thomsen

2012), most calcifiers are absent from areas naturally acidified by volcanic vents around Ischia, Italy, in oligotrophic waters (Hall-Spencer et al. 2008). Thus, there is evidence that food availability, and thus sufficient energy for increased metabolic rates, has the potential to overcome OA stress.

H5 - Stress effects will be carried over from parents to their F1 generation: It is an important task to consider carry-over effects when predicting the real effect OA could have on the organism's performance (Parker et al. 2012; Dupont et al. 2010; Sanford and Kelly 2011). Only very few OA studies have, yet, measured links between life-history stages or between adults and their offspring when determining a species' response to OA (but see Kurihara et al. 2008; Egilsdottir et al. 2009; Parker et al. 2009, 2010). Carry-over effects from barnacles' larval stages to post-larvae (juveniles) have been well documented under salinity stress (Thiyagarajan et al. 2002, 2003, 2007). From the present study I can, however, not confirm that OA based stress will be carried over from the larval to the juvenile phase. This is in line with investigations on the barnacle *A. amphitrite*, which did not show any OA based carry-over effects from the larval development to subsequent settlement and juvenile growth as well as on later egg production (McDonald et al. 2009). Here, I cannot either confirm that stress effects are carried over from the adults to their offspring. In contrast to the present study in which the different life-history stages were continuously exposed to OA, the freshly settled post-larvae in the study of Nasrolahi (2012) were immediately transferred from ambient salinities to stressful conditions of either reduced or increased salinities. Stress from transferring the sensitive post-larvae might herein be an important trigger driving the high mortality during 48 h post-settlement.

H6 - Barnacle populations from fluctuating pCO_2 environments are more tolerant to OA than barnacles from more stable pCO_2 habitats: If we want to understand the real potential impacts of future OA on marine organisms, it becomes important to study the response of a species over many generations. While this is possible for organisms with very short generations times (e.g. Collins and Bell 2004; Lohbeck et al. 2012), it remains difficult for organisms such as barnacles. Comparing the response of populations from habitats where OA occurs naturally, to less impacted areas would thus be intuitive to study. As shown for the Kiel Fjord (Thomsen et al. 2010) and in many shallow seas worldwide (e.g. Blackford and Gilbert 2007; Shim et al. 2007; Feely et al. 2008; Salisbury et al. 2008; Wootton et al. 2008; Miller et al. 2009; Feely et al. 2010), seasonal fluctuations in carbonate chemistry can be remarkable and local organisms might have adapted to cope with these fluctuations over many generations (Fabricius et al. 2011). Comparing different population from different habitats is difficult because of a lacking replication of the habitat as well as parameters, which cannot be controlled for. I cannot entirely confirm the hypothesis that barnacle populations from fluctuating pCO_2 environments are more tolerant to OA than barnacles from more stable pCO_2 habitats since larval stages of the barnacle *A. improvisus* were not impacted by OA in the Kiel as well as the Tjärnö population. However, I show that juvenile barnacles from the Tjärnö Archipelago respond more sensitively to OA than juvenile barnacles from the Kiel Fjord. Barnacle mortality was generally low in the present studies (compared to other species: Findlay et al. 2009; Findlay et al. 2010a, b) but 10-20 times higher in the Tjärnö population compared to the Kiel population. Survival and growth of barnacles from Tjärnö were impaired by OA while Kiel barnacles withstood severe OA well. The two different populations were not tested simultaneously under the same conditions and thus one need to be cautious with interpretations from the data. The data however support the hypothesis that barnacles from fluctuating habitats are more tolerant to OA than barnacles from more stable oceanic environments. Possibly, the Kiel population was pre-selected over many generations promoting OA tolerant genotypes (Schmidt and Rand 2001; Marshall et al. 2010; Sanford and Kelly 2011; Lohbeck et al. 2012). Nevertheless, additional factors such as salinity differ strongly between the habitats, what could act as an additional stressor (Nasrolahi et al. 2012, Nasrolahi 2012) and could have also affected the sensitivity of the different populations to OA.

4.5. Consequences on the Ecosystem Level

From the present study it becomes evident that future predictions for the barnacle *A. improvisus* are rather difficult because of diverging responses of different populations to simulated OA. Barnacles from the Kiel Fjord, however responded very weakly to OA; and from data including the entire life cycle of this species, *A. improvisus* will likely not to be strongly affected by future OA in this habitat. From many recent studies, it becomes evident that responses of marine organisms are species-specific (e.g. Kroeker et al. 2010) and that there will be losers and winners in an acidified ocean in the future, likely leading to community shifts (Fabricius et al. 2011; Hale et al. 2011). I will here discuss, in more detail, possible future changes on the ecosystem level for results obtained from OA (and other) studies over the last years from the Kiel Fjord habitat, with Kiel Fjord populations of key invertebrate species. The Kiel Fjord is characterized by strong seasonal fluctuations of the carbonate system (Thomsen et al. 2010) and estimates obtained from these data might not be extrapolated to the entire Baltic Sea ecosystem or to other ecosystems worldwide. Many other shallow enclosed areas such as for example the Eckernförde Bay (Vincent Saderne pers. commun.) are, nevertheless, characterized by similar features as was observed for the Kiel Fjord and organisms might show similar responses.

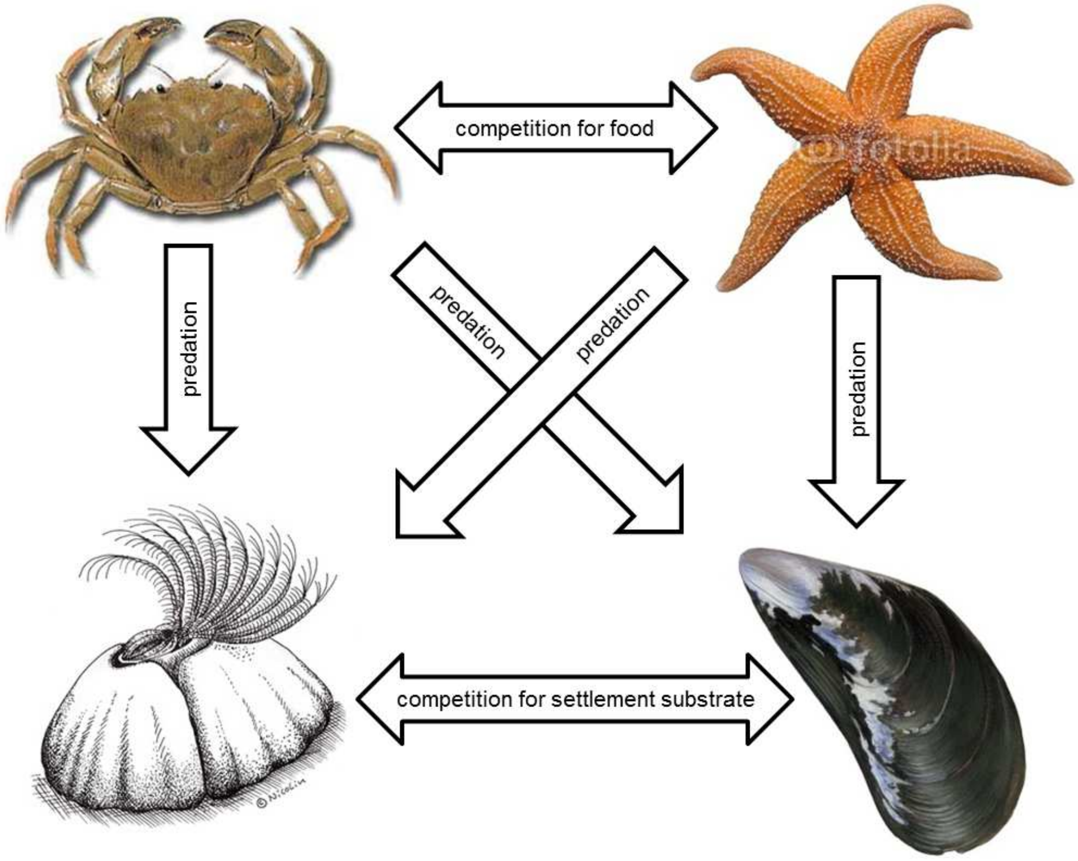


Figure 4.4: Simplified illustration of the interactions between two key-filter feeding invertebrates, the mussel *Mytilus edulis* and the barnacle *Amphibalanus improvisus* and their predator species *Carcinus maenas* and *Asterias rubens*. Picture were obtained from: www.fischgrosshandel-ahlen.de, www.livinginthebalticsea.com, www.masmar.net, www.ftcdn.net

The Baltic Sea is a relatively species poor ecosystem with few key-species being responsible for the major biomass production (HELCOM 2003). In the Kiel Fjord, the two benthic filter feeders - the mussel *Mytilus edulis* and the barnacle *Amphibalanus improvisus* - dominate large areas of the hard-bottom benthic ecosystem (Enderlein and Wahl 2004; Dürr and Wahl 2004). The two dominant predator species *Carcinus maenas* and *Asterias rubens* predate mainly on mussels (Appelhans et al. accepted in Marine

Ecology-Progress Series) but also on barnacles (Martin Paar, pers. commun.) and if one or the other of these key-species will be more impacted by future changes, shifts in the interactions between the species could have major impacts on the entire ecosystem (Enderlein and Wahl 2004, Reusch and Chapman 1997; Fig. 4.4).

In lab experiments as well in the field, *A. improvisus* showed a remarkable tolerance to OA, with likely no impacts on the overall recruitment success. This barnacle species strongly competes for settlement substrate with the blue mussel *M. edulis* (Thomsen 2012). Barnacle individuals settling in early summer are mainly overgrown by mussels over the later summer months and likely die due to reduced food availability or oxygen supply in dense aggregates of mussels (Thomsen 2012; Pansch pers. observ.). Juveniles and adults of the mussel *M. edulis* from the Kiel Fjord have been demonstrated to also tolerate high levels of $p\mathrm{CO}_2$ in lab experiments but, in general, reacted more sensitive to OA than barnacles (Thomsen 2012; Appelhans et al. accepted in Marine Ecology-Progress Series). Larval phases of this species and of closely related mussel species seem to be, however, more vulnerable to OA (Gazeau et al. 2010) with the possibility to affect the overall recruitment of this species. Nevertheless, field studies show that *M. edulis* can cope well with high-$p\mathrm{CO}_2$ seawater when the energy supply is abundant (Thomsen 2012). The barnacle *A. improvisus* was only competitive in less acidified seawater in regions, which also were characterised by a lower overall energy content. From personal observations I know that the barnacle *A. improvisus* can entirely dominate the habitat and outcompete mussels also under lower salinity values (Pansch pers. observ.). This can be for example observed in the Schwentine River inflow where salinities values can be reduced to below 10 and the Nord-Ostsee-Kanal with almost freshwater conditions (Nasrolahi and Pansch pers. observ.).

If an overall warming, desalination and increasing OA is considered with likely no changes in the food availability in the future (HELCOM 2003; IPCC 2007; The BACC author team 2008) for these key filter feeding species, mussels might be outcompeted by barnacles in the future. OA will however, not be the main driver but warming could promote *A. improvisus* (Nasrolahi et al. 2012; Nasrolahi 2012; present study). At the same time desalination and warming could negatively impact *M. edulis* (Hiebenthal 2009). While *A. improvisus* has been demonstrated to withstand significant seawater warming with rather positive effects of desalination on larvae (Nasrolahi et al. 2012; Nasrolahi 2012; present study), *M. edulis* suffered reduced growth rates under temperatures of 25°C and was also negatively impacted by desalination (Kossak 2006; Hiebenthal 2009). Thus, ecosystem changes seem possible when more than just a single stressor under "global climate change" is considered. However, precise predictions remain difficult from slightly variable effects of the interactions of these future changes upon different life-stages of the species.

Predictions on future ecosystems changes become even more difficult when the interactive effects of "global climate change" on the predator-prey interactions are considered. Predation by *A. rubens* and *C. maenas* largely controls the abundance of *M. edulis* in the Baltic Sea (Reusch and Chapman 1997; Laudien and Wahl 1999). Since both predators prefer relative small sized mussels as prey (Juanes 1992; Reusch and Chapman 1997; Leonard et al. 1999; Appelhans et al. accepted in Marine Ecology-Progress Series), faster growth and shell stability will allow *M. edulis* to earlier escape from predation (Elner 1978; Palmer 1981; Enderlein and Wahl 2004). Recent empirical data, however, show that direct OA effects on the prey (*M. edulis*) tended to be neutralized by concurrent effects on the predators (*A. rubens* and *C. maenas*; Appelhans et al. accepted in Marine Ecology-Progress Series). Nevertheless, since sea stars such as *A. rubens* are likely to be more impacted by OA than crustaceans such as *C. maenas* (Appelhans et al. accepted in Marine Ecology-Progress Series) community shifts are possible. Shifts in the preferred prey size under salinity stress as reported previously (Kossak 2006) further demonstrating the complexity of interactions and their possible changes in the future.

To this point, no data are available on the interactions of *A. improvisus* and its predators *A. rubens* and *C. maenas* under OA stress making it difficult to make further predictions. Predictions on the fate of barnacles or other organisms are also complicated by equally complex but mostly unstudied impacts of OA and warming on species interacting with larval stages such as for example the jellyfish *Mnemiopsis leidyi* predating on the barnacle nauplii (e.g. Javidpour et al. 2009).

5. Conclusions

Barnacles, in general, have been demonstrated to withstand predicted future OA well with mainly sub-lethal effects of different life stages under very severe OA scenarios. Nevertheless, there have been remarkable differences between barnacle populations of the same species, likely resulting from habitat differences and possible adaptation processes of barnacles or from range limits of certain species and thus additional stress such as reduced temperatures. While Tjärnö individuals of *A. improvisus* responded more sensitively to OA, individuals from Kiel were not as strongly impaired by OA. These population specific differences might be explained by the high natural variability in $p\mathrm{CO}_2$ and pH over the year and the possibility of barnacles to adapt to these fluctuations over more than 50 generations (considering a generation time of one year). Assuming that this potential for adaptation also is valid for other barnacle populations, *A. improvisus* from Tjärnö will likely adapt to anthropogenic OA in the future. Since OA is expected to increase more drastically in shallow coastal habitats in the future, the absolute OA-tolerance limits of this species are still to be evaluated in more detail. Additionally, the synergistic effects of OA and factors such as desalination need to be considered when future predictions are made.

From the present study as well as from earlier investigations it becomes evident that OA driven responses of barnacles are largely depending on the species and on the population investigated. Additionally, seasonal variation in the response of barnacles to OA (from differences between single experiments of the same population) may play an important role, as the present study demonstrates that a cohort of *A. improvisus* individuals collected in autumn suffered from OA while barnacle cohorts collected in early summer were not impaired by OA. This hypothesis needs to be investigated in more detail but suggests that summer cohorts of barnacles might be more resilient to OA than autumn cohorts.

Barnacles live in highly fluctuating habitats sub-tidally or even in the intertidal and might thus be pre-selected to withstand strong fluctuations in their natural environment. I assume that organisms, which experience strong shifts in the environmental parameters, even on a daily basis, might be more tolerant to future OA than organisms from more stable habitats. Nevertheless, since community structures depend on the responses of various organisms and it has been widely demonstrated that there will be “losers” and “winners” under OA, which will likely lead to changes in community structures in a future acidified ocean. Economic, cultural and societal dependencies on the world’s oceans make this an important topic to be discussed. Disrupting the oceanic system could have severe consequences for humankind. Seafood recourses and tourisms are major issues potentially impacted by these “global climate changes” in a future ocean and we should not underestimate the long-term effects that stressors such as warming and OA will have on the marine ecosystem.

6. Looking Ahead

Almost all projections of the effects of OA on marine ecosystems assume that responses measured in present-day populations, often in short-term experiments, will apply to future populations (but see e.g. Collins and Bell 2004; Lohbeck et al. 2012). This approach overlooks the potential for evolutionary processes. Short-term, (and even long-term within-generation) responses to environmental shifts mainly reflect physiological acclimation capacities, i.e. plasticity of individual organisms (Pigliucci 2001). In contrast, trans-generational responses more realistically reflect the ecological and evolutionary impacts of environmental shifts and only trans-generational experiments allow quantification of the capacity of populations to adapt to a changing environment (Sunday et al. 2011).

Species biodiversity (i.e. species richness) has been suggested to provide "insurance" of ecosystem function against environmental shifts (Loreau 2000). Analogously, genetic diversity within species (intraspecific diversity) has been suggested to have similar "insurance" effects (Gamfeldt et al. 2005), although this latter hypothesis has been tested only rarely in marine species. In a novel experimental setting, Gamfeldt et al. (2005) showed intraspecific diversity to increase the performance of barnacles. High genetic diversity also enhanced seagrass community resistance to disturbance by grazing geese (Hughes and Stachowicz 2004) and increased resistance to thermal stress in seagrass meadows (Reusch et al. 2005). For the great majority of marine invertebrates, however, we know nothing about the capacity for genetic variation within populations to provide insurance against climate change.

Given that prior environmental history is likely to influence the plasticity and genetic diversity of populations with respect to future environmental shifts, it can be predicted that the plasticity and genetic diversity of local populations to future OA will be related to natural variation in seawater $p\text{CO}_2$ (Johannesson et al. 2011). Coastal and estuarine ecosystems are dominated by characteristic taxa such as mussels, seaweeds and barnacles, all of which have been the focus of intense research over the last 50 years. Globally barnacles are ecologically important ecosystem engineers and are a vital ecosystem component within the species poor Baltic Sea (Berntsson and Jonsson 2003).

The main goal for future research should be to determine whether the strong selective pressure represented by near-future ocean acidification will overwhelm the adaptation potential of marine invertebrates. A key focus should be whether adaptation potential by means of genetic diversity correlates with habitat related natural pCO_2 variability. The barnacle *Amphibalanus improvisus* could in this case ideally used as a model organism.

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CURRICULUM VITAE

Christian Pansch

Feldstraße 102, 24105 Kiel, Deutschland
Geboren am 23. Juni 1981 in Stendal
Staatsangehörigkeit: deutsch

BERUFLICHER WERDEGANG

Seit Apr 2008	Promotionsstudent und wissenschaftlicher Mitarbeiter in der Benthos-Ökologie am GEOMAR in Kiel zum Thema der Auswirkungen von Umweltstress auf frühontogenetische Lebensstadien von *Amphibalanus improvisus* unter der Betreuung von Prof. Martin Wahl
Nov 2007	**Erworbene Qualifikation als Diplom Biologe („sehr gut“):** Öffentlicher Vortrag der Diplomarbeit (Verteidigung)
Okt 2006 – Nov 2007	**Diplomarbeit:** “The effects of light stress on the capacity of macroalgae to defend themselves against grazing and fouling” unter der Betreuung von Prof. Martin Wahl, Dr. Mark Lenz und Prof. Martin Thiel im Rahmen des stipendien-geförderten GAME-Projekts am GEOMAR in Kiel. Feld- und Laborarbeiten fanden an der Universidad Católica del Norte in Coquimbo, Chile statt (Nov 2006 – Apr 2007)
Aug 2005 – Jan 2006	**Erasmus Austausch an der Universität Uppsala, Schweden:** “Biology and Evolution of Insects”, “Population Biology” (30 ECTS)
Apr – Jun 2005	**Auslandspraktikum (Muschelfarm, Irland):** Aufzucht von Jakobsmuscheln
Okt 2001 – Nov 2007	**Biologiestudium an der Universität Rostock** Hauptfach: Zoologie**;** Nebenfächer: Meeresbiologie, Tierphysiologie, Meeresgeologie (Universität Greifswald)
Jun – Sept 2001	Vermessungsarbeiten bei der Firma Johann Bunte GmbH & Co
Jul 2000 – Mai 2001	**Zivildienst:** Rettungssanitäter im Rettungsdienst und Krankentransport (Ausbildung zum Rettungssanitäter)
Jul 1992 – Jul 2000	Bismarck Gymnasium Genthin (Abschluss zur allgemeinen Hochschulreife)
Jun 1988 – Jun 1992	Grundschule Jerichow

Christian Pansch Kiel, den 08.06.2012

ERKLÄRUNG

Christian Pansch, Feldstraße 102, 24106 Kiel

Erklärung gem. § 9 Promotionsordnung

Ich erkläre hiermit,
a) dass die Abhandlung - abgesehen von der Beratung durch die Betreuerin oder den Betreuer - nach Inhalt und Form meine eigene Arbeit ist,
b) dass die Arbeit nicht schon einer anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegen hat, veröffentlicht worden ist oder zur Veröffentlichung eingereicht wurde;
c) dass die Arbeit unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft entstanden ist.

Teile dieser Arbeit sind bereits veröffentlicht, wurden zur Veröffentlichung in Fachzeitschriften eingereicht oder sind in Vorbereitung eingereicht zu werden:

Pansch C, Nasrolahi A, Appelhans YS, Wahl M (2012) Impacts of ocean warming and acidification on the larval development of the barnacle *Amphibalanus improvisus*. Journal of Experimental Marine Biology and Ecology, 420–421, 48-55: *CP und MW haben die Studie entworfen, CP und AN haben die Daten erhoben, CP hat die Daten analysiert und das Manuskript eigenständig geschrieben; alle Koautoren haben weiterhin beim Überarbeiten des Manuskripts mitgewirkt.*

Pansch C, Schlegel P, Wahl M, Havenhand JN (prepared for submission in Marine Ecology-Progress Series) Larval development of the barnacle *Amphibalanus improvisus* in an acidified ocean: *CP, PS, MW und JNH haben die Studie entworfen, CP und PS haben die Daten erhoben, CP und PS haben die Daten analysiert und CP hat das Manuskript eigenständig geschrieben; alle Koautoren haben weiterhin beim Überarbeiten des Manuskripts mitgewirkt.*

Pansch C, Nasrolahi A, Appelhans YS, Wahl M (under review in Marine Biology) Ocean warming and ocean acidification - impacts on juvenile *Amphibalanus improvisus. CP und MW haben die Studie entworfen, CP und AN haben die Daten erhoben, CP hat die Daten analysiert und das Manuskript eigenständig geschrieben; alle Koautoren haben weiterhin beim Überarbeiten des Manuskripts mitgewirkt.*

Pansch C, Schaub I, Havenhand JN, Wahl M (prepared for submission in Global Change Biology) Do habitat traits and food availability modulate the impact of ocean acidification on juvenile *Amphibalanus improvisus*: *CP, IS und MW haben die Studie entworfen, CP und IS haben die Daten erhoben und analysiert und CP hat das Manuskript eigenständig geschrieben; alle Koautoren haben weiterhin beim Überarbeiten des Manuskripts mitgewirkt.*

Christian Pansch Kiel, den 08.06.12

Und was von allem bleibt ist die Seepocke

Nach Martin Brookes