Effects of Ocean Acidification on Atlantic Cod Larvae (Gadus morhua)

Dissertation
zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften

an der **Mathematisch-Naturwissenschaftlichen Fakultät**
der **Christian-Albrechts-Universität zu Kiel**

Martina H. Stiasny

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vorgelegt von

Martina Hanneliese Stiasny

Kiel, August 2017

Gutachter: Prof. Dr. Thorsten Reusch

Gutachter: Prof. Dr. Oscar Puebla

Tag der Disputation: 7.November 2017

Zum Druck genehmigt: 7.November 2017

I think fish is nice,
But then I think that rain is wet,
So who am I to judge?
Douglas Adams

SUMMARY

Throughout the twentieth and the beginning of the twenty-first century technical advancements in many industries as well as the vast increase in world population have lead to increasing emissions of greenhouse gases like carbon dioxide. At the same time carbon sinks like forests have partly disappeared due to altered land uses. The changes in the chemistry of the atmosphere not only result in retention of heat causing global warming, but also transfer to the oceans. The oceans take up a substantial amount of anthropogenic carbon dioxide. This buffers the climate of the earth, but has negative consequences for marine life. The world's oceans are not only warming, but are furthermore acidifying through the reaction of seawater with carbon dioxide, which releases hydrogen ions, measured in pH. This process, termed ocean acidification, threatens many marine organisms.

Evidence of impacts of acidification on many marine organisms and ecosystems has accumulated over the last few decades. Nevertheless there are still vast knowledge gaps, particularly for the more complex species or changes relating to the population level. Most commercial fish species are near the top of the food web and are therefore keystone species in the ecosystems. Even though reliable knowledge for these species is of particular importance, since they not only support the livelihood of many people and assist in food security, but also need to be managed sustainably, it is still largely missing. Good management relies on a good scientific understanding of these species as well as dependable quantitative data on population processes, like recruitment and growth.

The aim of this thesis was to provide greater understanding of the impact of ocean acidification on one of the most important commercial species, the Atlantic cod (*Gadus morhua*) and to provide a quantitative foundation to evaluate recruitment processes of this species. Most stocks of the Atlantic cod have been under substantial fishing pressure since the onset of industrialized fishing. Overfishing and even stock collapses have occurred in the past. Recently there are also some success stories of sustainable management successes and recoveries. However, it remains unclear if these populations are resilient enough to additional stressors through climate change.

This thesis has quantified the effect of ocean acidification on larval survival of two Atlantic cod stocks from the Western Baltic Sea and the Barents Sea and how this translates into the recruitment of these populations. Ocean acidification levels were chosen to reflect carbon dioxide concentrations as they are expected to occur at the end of the century. Results for both stocks show significantly reduced survival under ocean acidification. This may result in dramatically decreased recruitment.

Additionally, the effect of acidification and its interaction with food availability on larval growth was quantified for the Barents Sea cod. The food treatment significantly changed the effect of the acidification treatment. Larvae, which are not energy limited through a high food treatment, showed no effect of acidification on growth. Larvae in the low food treatment on the other hand showed an increase in growth under acidification. These larvae also exhibited increased ossification of the vertebrae, but also an increased amount of organ impairments, particularly in the liver and the eyes.

In order to investigate the potential for adaptation or acclimation, it was furthermore explored whether the acclimation of the parental generation to acidification had a significant effect on larval survival and organ development. The effect of parental acclimation again depended on the food available to the larvae. Under ideal conditions for the offspring, parental acclimation had a buffering effect on larval survival, however this was reversed under food limitation.

The results of this dissertation demonstrate that ocean acidification may pose a severe threat to Atlantic cod populations. Nonetheless, the exact effects are very complex and rely on other factors, like the exposure of the parental generation to acidification and on food availability to the larvae.

ZUSAMMENFASSUNG

Im Verlauf des zwanzigsten und dem Beginn des einundzwanzigsten Jahrhunderts haben der technische Fortschritt in Verbindung mit einem starken Zuwachs in der Weltbevölkerung zu einem hohen Anstieg der Emissionen von Treibhausgasen wie Kohlenstoffdioxid geführt. Gleichzeitig verschwinden Kohlenstoffsenken wie Wälder durch geänderte Landnutzungen zunehmend. Änderungen in der Chemie der Atmosphäre führen nicht nur zu Wärmerückhaltung und Klimawandel, sondern auch zu Änderungen in der Meereschemie. Ozeane, die einen substantiellen Teil des anthropogenen Kohlenstoffdioxids aufnehmen, puffern das Weltklima. Dies hat jedoch negative Konsequenzen für das marine Leben. Die Weltmeere erwärmen sich nicht nur sondern versauern auch durch die Reaktion des Seewassers mit Kohlenstoffdioxid. Dadurch werden in pH gemessene Wasserstoffionen freigesetzt. Dieser Prozess, genannt Ozeanversauerung, bedroht marine Organismen.

In den letzten Jahrzehnten häufen sich die Hinweise auf den Einfluss von Ozeanversauerung auf marine Organismen und Ökosysteme. Trotzdem gibt es weiterhin große Wissenslücken, insbesondere im Hinblick auf komplexe Arten und Auswirkungen auf ganze Populationen. Die meisten kommerziellen Fischarten stehen nahe der Spitze des Nahrungsnetzes und sind daher Schlüsselarten im Ökosystem. Obwohl verlässliches Wissen für diese Arten von besonderer Bedeutung ist, da sie die Grundlage des Lebensunterhaltes für viele Menschen bilden, zur Lebensmittelsicherheit beitragen und nachhaltig gemanagt werden müssen, ist dieses häufig nicht verfügbar. Gutes Management basiert auf fundiertem, wissenschaftlichen Wissen so wie auf verlässlichen, quantitativen Daten zu Prozessen auf der Populationsebene, zum Beispiel Rekrutierung und Wachstum.

Das Ziel dieser Dissertation ist ein besseres Verständnis des Einflusses von Ozeanversauerung auf eine der kommerziell genutztesten Fischarten, den Atlantischen Dorsch (*Gadus morhua*), zu erreichen und eine quantitative Basis zu legen um Änderungen in den Rekrutierungsprozessen dieser Art zu bewerten. Die meisten Bestände des Atlantischen Dorschs unterliegen seit Beginn der industriellen Fischerei starkem Fischereidruck. In der Vergangenheit sind bereits Überfischung und selbst Zusammenbrüche einiger Bestände aufgetreten. In jüngster Vergangenheit gab es auch Erfolgsgeschichten von nachhaltigem Management und Bestandserholungen. Trotzdem bleibt es fraglich, ob Bestände belastbar genug sind dem zusätzlichen Druck durch Klimawandel stand zu halten.

Diese Dissertation quantifiziert den Effekt von Ozeanversauerung auf das Überleben von Larven von zwei Populationen des Atlantischen Dorschs aus der westlichen Ostsee und der Barentssee und zeigt, wie sich dieses auf die Rekrutierung auswirkt. Getestet wurde eine Versauerung, wie sie bis zum Ende dieses Jahrhunderts zu erwarten ist. Die Ergebnisse für beide Bestände zeigen stark

verminderte Überlebensraten durch Versauerung, was dramatische Auswirkungen auf die Rekrutierung haben kann.

Zusätzlich wurden die Auswirkungen von der Ozeanversauerung und die Wechselwirkungen mit Nahrungsverfügbarkeit auf das Larvenwachstum des Barentssee Bestandes quantifiziert. Die experimentelle Nahrungsbehandlung veränderte den Effekt der Versauerung signifikant. Larven, die durch viel Nahrung nicht Energie limitiert waren, zeigten durch die Versauerung des Wassers keine Auswirkung auf das Wachstum. Larven, denen weniger Nahrung zur Verfügung gestellt wurde, wiesen ein stärkeres Wachstum durch die Versauerung auf. Diese Larven hatten darüber hinaus eine stärker ossifizierte Wirbelsäule, aber auch ein erhöhtes Vorkommen von Organschädigungen, besonders in der Leber und in den Augen.

Um das Potential zur Adaptation oder Akklimatisierung der Larven zu untersuchen, wurden die Auswirkungen einer Akklimatisierung der Elterngeneration an die Versauerung auf das Überleben der Larven und ihrer Organentwicklung ermittelt. Auch dabei spielte die Nahrungsverfügbarkeit eine wesentliche Rolle. Unter idealen Bedingungen für die Larven erzeugte die Akklimatisierung der Eltern einen puffernden Erfolg auf das Überleben der Larven. Das Gegenteil war allerdings der Fall wenn die Larven nahrungslimitiert waren.

Die Ergebnisse dieser Dissertation belegen, dass Ozeanversauerung eine große Bedrohung für die Bestände des Atlantischen Dorsches darstellen kann. Nichtsdestotrotz sind die genauen Auswirkungen sehr komplex und hängen von vielen Faktoren ab, wie beispielsweise die Anpassung der Elterngeneration an die Ozeanversauerung und die Nahrungsverfügbarkeit der Larven.

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1 INTRODUCTION

1.1 Climate Change and Ocean Acidification

Worldwide emissions of greenhouse gases, in particular carbon dioxide, have consistently increased since the industrialization and are still increasing, caused by anthropogenic processes such as burning of fossil fuels and altered land uses (Sabine *et al.*, 2004; IPCC, 2013) (Figure 1.1). These gases are causing significant changes in the physical properties of the atmosphere, particularly the retention of heat leading to the greenhouse effect, ultimately resulting in global warming. Atmospheric carbon dioxide concentrations have increased by 40% since pre-industrial levels and are now at the highest concentrations of at least the past 800 000 years (IPCC, 2013). Global atmospheric CO_2 concentrations reached 410 ppm in 2017 at Mauna Loa Observatory (Keeling Curve, Scripps Institution of Oceanography). The process of global warming is in part buffered by the oceans, which absorb about a quarter to a third of the access carbon dioxide, thereby slowing the warming of the atmosphere and concurrently also the oceans.

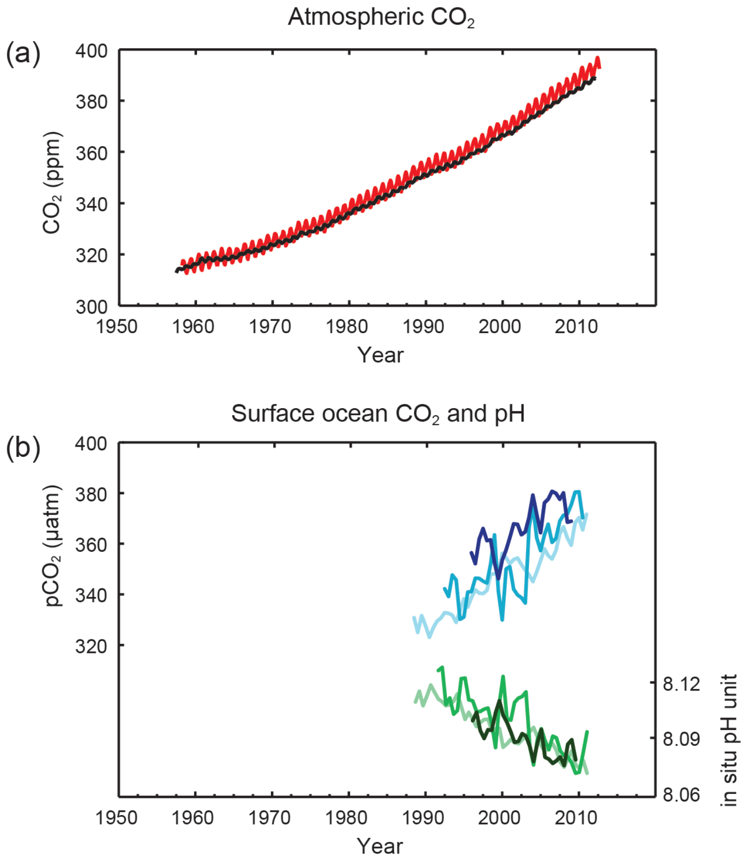

Figure 1.1. Multiple observed indicators of a changing global carbon cycle: (a) atmospheric concentrations of carbon dioxide (CO_2) from Mauna Loa (19°32'N, 155°34'W – red) and South Pole (89°59'S, 24°48'W – black) since 1958; (b) partial pressure of dissolved CO_2 at the ocean surface (blue curves) and in situ pH (green curves), a measure of the acidity of ocean water. Measurements are from three stations from the Atlantic (29°10'N, 15°30'W – dark blue/dark green; 31°40'N, 64°10'W – blue/green) and the Pacific Oceans (22°45'N, 158°00'W – light blue/light green). Taken from (IPCC, 2013).

However, the uptake of CO_2 by ocean water comes at a price, namely ocean acidification, the "other CO_2 problem" (Doney *et al.*, 2009). In seawater carbon dioxide (CO_2) reacts with the water molecules (H_2O) to form carbonic acid (H_2CO_3), which dissociates further into bicarbonate ions (HCO_3^-) and carbonate ions (CO_3^{2-}) releasing hydrogen ions (H^+). This increase in hydrogen ions is measured as a decrease in pH (Feely *et al.*, 2004; Caldeira & Wickett, 2005; Doney *et al.*, 2009). The ocean surface waters have shown a decrease in pH of 0.1 since the beginning of industrialization. (Figure 1.1) This corresponds to an increase of hydrogen ions of 26%. (IPCC, 2013) Globally the ocean surface waters are projected to experience a further decrease in pH between 0.1 and 0.4 by the year 2100 depending on the assumed reaction concentrations pathway of the Intergovernmental Panel on Climate Change (IPCC) (Figure 1.2).

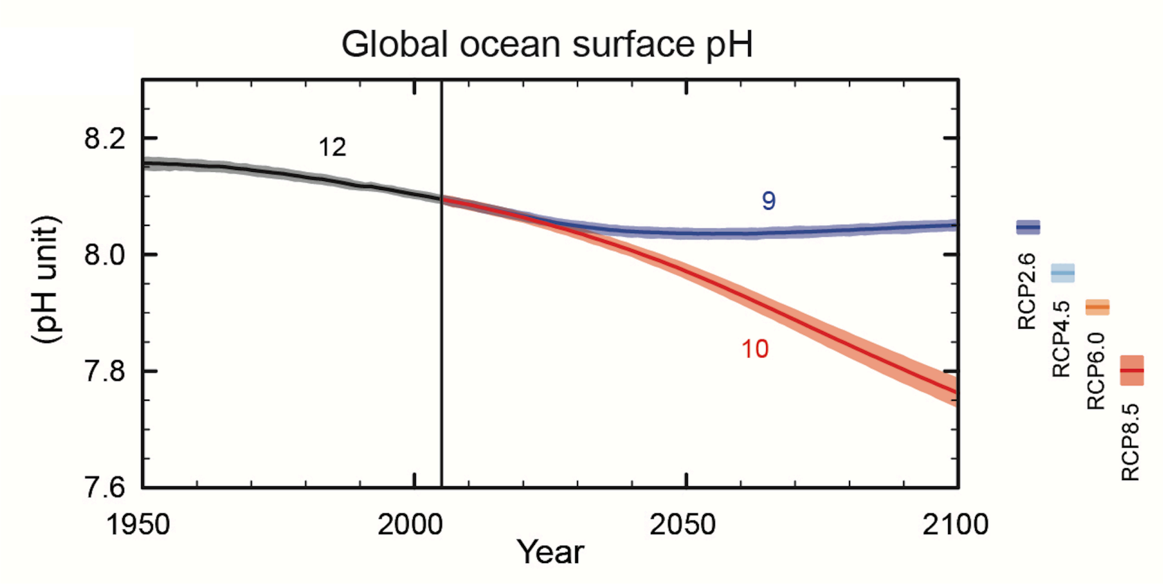

Figure 1.2. Simulated time series from 1950 to 2100 for global mean ocean surface pH. Time series of projections a measure of uncertainty (shading) are shown for IPCC scenarios RCP 2.6 (blue) and RCP 8.5 (red). Black (grey shading) is the modelled historial evolution using historical reconstructed forcings. The mean and associated uncertainties averages over 2081-2100 are given for all RCP scenarios as colored vertical bars. Taken from (IPCC, 2013)

In order to explore the biological effects of certain species it is important to understand the regional dynamics of ocean acidification. High latitudes are expected to experience lower pH values than the global average and different seasonal cycles. This is due to several different processes. Firstly carbon dioxide is more soluble at lower temperatures, so the surface waters take up more carbon dioxide. This is unfortunately positively enforced by the melting of sea ice, which uncovers greater areas of the ocean allowing more interaction with the atmosphere.

At high latitudes, pH values also only exhibit small diurnal cycles, because of stable light conditions, therefore presenting more constant stress. At the same time seasonal signals become more significant with particularly low pH values during very early spring (Kaltin *et al.*, 2002). Unfortunately this corresponds to the spawning time of many commercial species, such as the target species of my dissertation Atlantic cod (*Gadus morhua*), which spawn before the first phytoplankton blooms of the year.

Coastal areas, in which Atlantic cod spawn, are additionally likely to experience higher levels of acidification than the open ocean since the input of terrestrial organic carbon, which is oxidized in the water by photolytic or microbial oxidation, produces further CO_2. Melzner et al. (2012) show that coastal areas in e.g. the Baltic may experience drastic variations and much lower pH values due to excess oxygen consumption and local upwellings.

The Atlantic cod is therefore due to its natural distribution and spawning behaviour particularly prone to experience acidification levels far exceeding global averages. This thesis focuses on two Atlantic cod stocks. The Arcto-Norwegian cod is spawning along the coast of Northern Norway (Sundby & Nakken, 2008) and is distributed around the Southern Barents Sea. Projected changes in pH for this area are a decrease in pH down to values around 7.75 at the end of this century (Figure 1.3) (Denman *et al.*, 2011; AMAP, 2013).

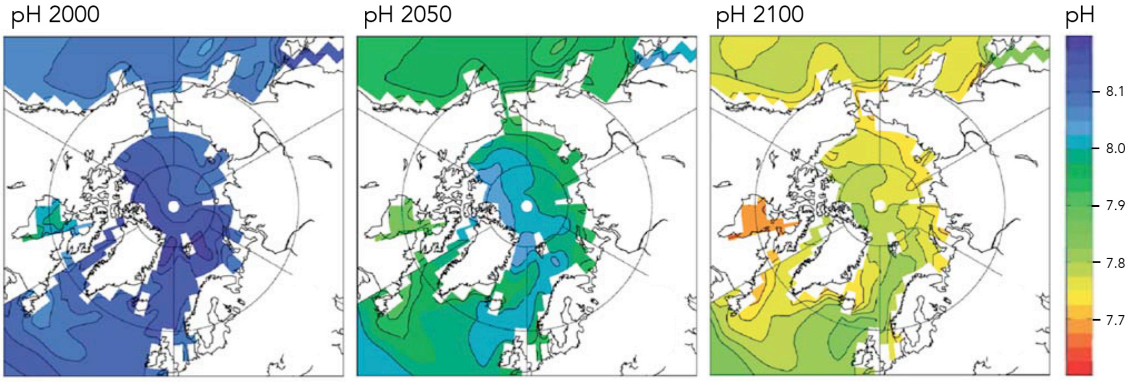

Figure 1.3. Projected change in pH in the Arctic through the 21st century after (Denman *et al.*, 2011), taken from the Arctic Monitoring and Assessment Programme Assessment 2013: Arctic Ocean Acidification (AMAP, 2013)

The Western Baltic Stock is distributed throughout the Western Baltic Sea. It is in the unfortunate situation of having a comparatively huge terrestrial catch area compared to its own water volume with a large human population living on its coasts. Water exchange with adjacent seas, in this case the North Sea through the Skagerrak and Kattegat is minimal. It is therefore under unparalleled anthropogenic pressures. The pH in the Baltic is already lower than most waters and projected acidification levels far exceed the global predictions. Strong eutrophication in this region leads to high primary productivity, which naturally reduces the pH. The change in pH in the Western Baltic may reach -0.2 to -0.3 even in the scenario with lowest emissions (B1) (Figure 1.4).

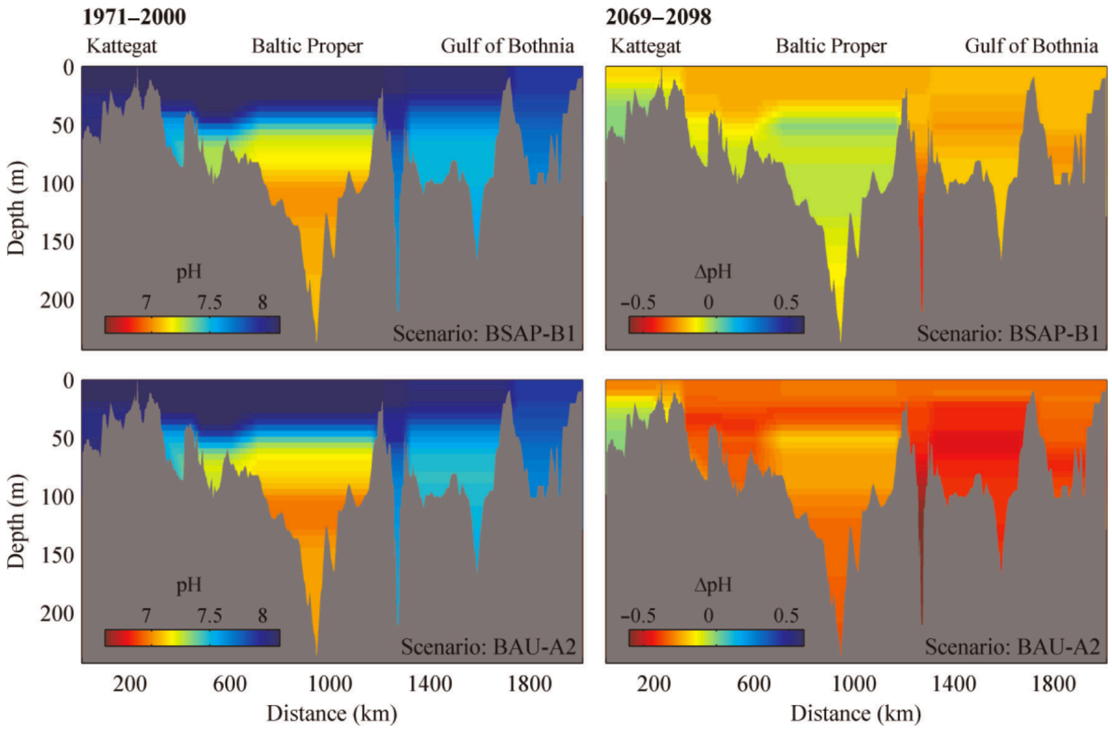

Figure 1.4. Current pH (1971-2000) and scenario pH changes (2069-2098) along a Baltic Sea transect for two scenarios. Taken from (Omstedt *et al.*, 2012). Please refer to paper for details.

1.2 Ocean Acidification effects on the physiology of marine fishes and their early life stages

In marine fishes acid-base regulation is taking place mainly at the gill-water surface. It is linked to the excretion of carbon dioxide, since CO_2 can be hydrated to H^+ and HCO_3^- in a reversible reaction, which is catalyzed by the enzyme carbonic anhydrase (CA). Water-breathing fish are at a disadvantage to regulate their metabolic acid-base balance compared air-breathing animals, because of their low arterial partial pressure of CO_2. Any environmental conditions, such as increased CO_2 concentrations of the seawater, which further lower the CO_2 excretion potential, will result in respiratory acidosis and the need to actively excrete hydrogen ions. This happens primarily across the gills. (Perry & Gilmour, 2006) The excretion of H^+ is coupled with a Na^+ influx, facilitated by the Na^+/H^+ exchanger. Similarly HCO_3^- is coupled with an efflux of Cl^-. (Figure 1.5) The energy is provided by the Na^+/K^+ ATPase, which provides the necessary gradients across which the exchangers can function. Cl^- is moved to the seawater through chloride channels in order to maintain electroneutrality, while the bicarbonate is transferred to the extracellular fluid. (Perry & Gilmour, 2006; Melzner *et al.*, 2009a)

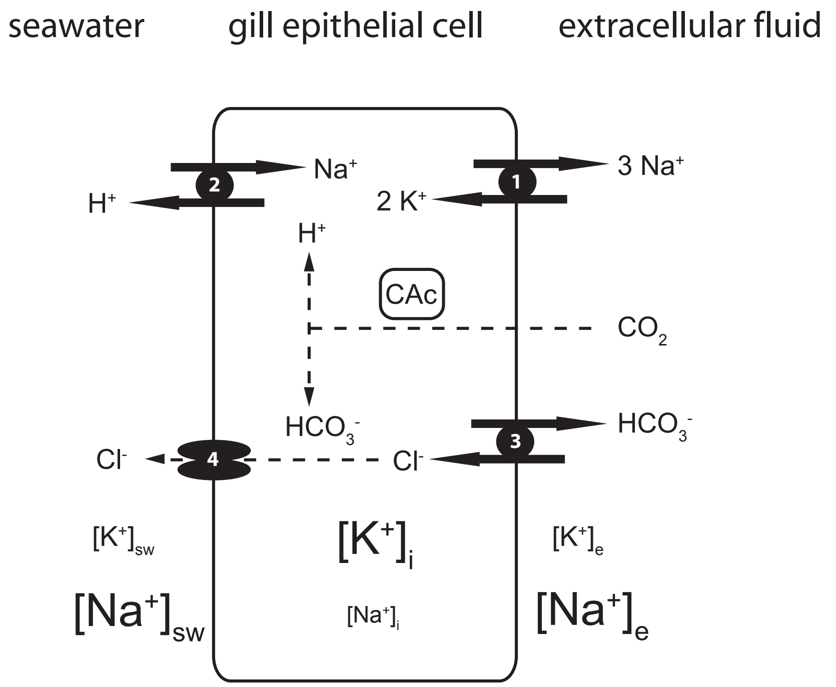

Figure 1.5. Simplified schematic depiction of an epithelial gill cell (ionocyte) of a teleost fish (taken from (Melzner *et al.*, 2009a) (adapted from (Perry & Gilmour, 2006)) (1) =Na^+/K^+ ATPase, (2)=Na^+/H^+ exchanger, (3)=$Cl^-/HCO^-{}_3$ exchanger, (4)=Cl^- channel (e.g. CFTR), CAc = cytoplasmic carbonic anhydrase.

The buffering of a decrease in pH is therefore always linked to an increase in bicarbonate ions. The relationship between partial pressure of CO_2, extracellular pH, and extracellular HCO_3^- concentrations can be seen in the Davenport diagram in Figure 1.6. The green line represents an organism with full pH compensation through active bicarbonate accumulation, like a marine fish. Any pCO_2 represents a definite combination of concentrations of HCO_3^- and pH. The extracellular pH may be lowered in the short term, but HCO_3^- concentrations start to rise immediately and after some time, the pH is close to its initial value. However, it is important to note that while pH returns to ideal conditions, the bicarbonate concentrations and the pCO_2 have changed dramatically.

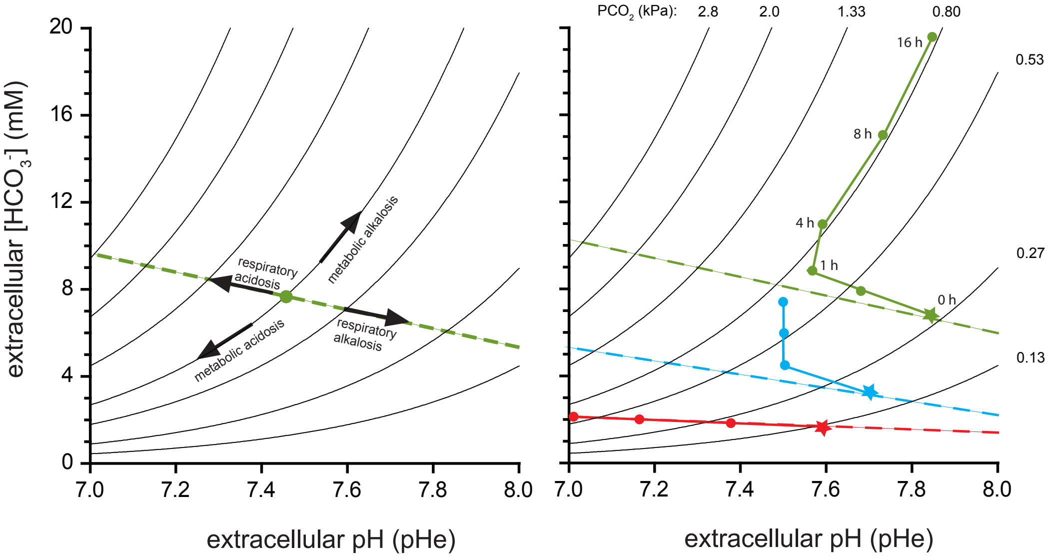

Figure 1.6. Davenport diagrams. (A) Schematic illustration of non-bicarbonate buffer line, dashed green line. Arrows indicate changes in pCO_2 and $[HCO_3^-]$ during respiratory acidosis/alkalosis and metabolic acidosis/alkalosis. (B) Three different hypothetical organisms subjected to 0.5 kPA (ca. 4900 µatm) environmental hypercapnia. Red symbols: No active accumulation of bicarbonate in the extracellular space to compensate pH. pH follows the non-bicarbonate buffer line. Blue symbols, green symbols: partial/full pH compensation through active bicarbonate accumulation. Stars indicate control parameters, numbers indicate time (h) exposed to elevated pCO_2 (hypothetical time course!). Taken from Melzner *et al.* (2009a)

Although pH compensation is possible in fish, it entails a changed blood composition. Importantly, since this is driven ultimately by the Na^+/K^+ ATPase, it is an energetically costly process. The exact physiological mechanisms are still unknown. Moreover, how the response of early life stages might differ from the above-explained mechanisms is yet largely uncertain. Egg and early larval stages of marine fish do not have fully functional gills yet, which might lower their compensatory potential, while they are more vulnerable to environmental change through larger surface to volume relationships and more permeable surfaces. However, it is believed that they have the capacity, though possibly less efficient, to regulate their internal pH as well, through chloride cells and possibly other channels across the whole body surface (Falk-Petersen, 2005). It is reasonable to assume though that regulation may be less efficient and the stress due to changes in blood composition and the increased energy demand weighs particularly heavy during development.

1.3 The ecology of Atlantic Cod (*Gadus morhua*) and its fisheries

The Atlantic cod (*Gadus morhua*, Linnaeus, 1758) is a demersal species with a geographical distribution spanning most of the Northern Atlantic Ocean. It is most often found in depths between 150 to 200 m on the continental shelf, but may be seen in depths of more than 600 m. Adult cod can tolerate a wide range of conditions in terms of temperature and salinity from arctic conditions to nearly 20°C and from nearly freshwater to oceanic waters. However, early life stages rely on a far narrower niche of conditions. Spawning only occurs below 12°C and the egg stages rely on the right salinity to stay buoyant within the water column. In the open ocean this is found close to the surface, while in the Baltic Sea eggs are found just below the halocline (Nissling & Westin, 1997). In roughly the first week after hatching larvae rely on their yolk sac for nutrition and stay in the water column (Heath & Lough, 2005). Once the larvae start feeding they become more mobile. Preferred prey items are early life stages of copepods (Seljeset *et al.*, 2010; Ottersen *et al.*, 2014).

Atlantic cod is one of the most important commercial species in the Northern Atlantic (FAO, 2016). Most stocks are overexploited, including the Western Baltic cod stock. Advice from the International Council for the Exploration of the Sea (ICES) has suggested large cuts in total allowable catches in recent years. The cod stock in the Barents Sea is considered to be one of the success stories of sustainable management. Newly established harvest control rules were however also likely supported by favourable temperatures (Kjesbu *et al.*, 2014). Nonetheless, the latest advice from ICES has suggested a strong reduction in catches due to very low recruitment over the last decade (ICES, 2017).

1.4 Atlantic cod and ocean acidification – the previous state-of-art

The results of few studies are available on the effect of ocean acidification on Atlantic cod. Cod larvae from the Eastern Baltic were shown to be largely robust in terms of survival, hatching, development and otolith size even under very high CO_2 concentrations of 4000 ppm (Frommel *et al.*, 2013). No comparative study had been done on the Western Baltic stock. The Norwegian coastal cod on the other hand has been shown to be susceptible to very high concentrations of CO_2. Severe tissue damage was found in the larvae at 1800 and 4200 µatm CO_2 (Frommel *et al.*, 2012). Unfortunately this study wasn't able to follow the survival of these larvae. The absence of impaired larvae towards the end of the experiment suggested an increased mortality, but the data is lacking. The swimming kinematics of these larvae were not shown to be altered by the acidification treatment (Maneja *et al.*, 2012), but larvae from the high CO_2 treatments showed an increase in otolith size (Maneja *et al.*, 2013). Juvenile Atlantic cod under long term carbon dioxide exposure showed no alterations in swimming performance, but at very low pH values of around 7.01 an increase in Na^+/K^+-ATPase protein expression and elevated Na^+/K^+-ATPase activity was shown (Melzner *et al.*, 2009b), which proves that the cod regulated their enzymatic capacity to cope with the CO_2 stress.

1.5 The importance of survival and growth measurements

This dissertation intentionally focused on the fundamental parameters of survival and growth of the larvae in order to build the basis for up-scaling efforts to the fished population. Survival and growth form the basis for any population model.

Additionally, samples to measure physiological impairments are most often taken from surviving individuals. These represent the most successful individuals being able to tolerate CO_2 stress, since natural mortality in these mass spawners is extremely high, even without taking predation into account. It is therefore imperative that differences in survival between treatments are taken into account, when discussing all other changes. Moreover, without measuring survival, it is impossible to judge whether samples were taken at significant periods of time or whether they signify some kind of end point in the development. Survival measurements are therefore needed as a general framework to put all other data into perspective.

Most studies so far have been ignoring survival as a response measurement or have relied on a final measurement of survival at the end of the experiment. This is insufficient. Only regular measurements throughout the experiment, which allow for the calculation of a survival curve, can demonstrate the times of greatest mortalities and the time when survival has reached a more stable state. Only survival curve allows for projections on the quantitative effect of ocean acidification on recruitment to the population.

Similarly growth parameters are fundamental to judge the general development of the organisms. Survival and growth data are needed to review whether the larvae in the experimental set-up developed as needed and planned.

1.6 Aim of this dissertation

The aim is to provide a good quantitative basis for integration of physiological effects into population dynamics. Modelling efforts to explore the effects of acidification on populations and stocks need to be based on reliable quantitative biological data, which has so far been missing for the Atlantic cod, as well as for most other commercial species. The focus of this thesis was therefore put on the best possible quantification of survival and growth during the larval stage, which is one of the most important bottlenecks in recruitment.

Additionally, the aim of this dissertation is to further our understanding of the effects of ocean acidification on the physiology of cod larvae under realistic acidification scenarios and to explore the interaction with food availability. Furthermore, the effect of parental acclimation to acidification was tested.

1.7 Thesis outline

This thesis contains three chapters, which are based on three separate manuscripts, listed under 1.7.1. The first manuscript entitled "Ocean Acidification Effects on Atlantic Cod Larval Survival and recruitment to the fished population" describes the effect of ocean acidification on survival and recruitment of two Atlantic cod stocks, namely from the Western Baltic and from the Barents Sea. Carbon dioxide concentrations as they are expected to occur at the end of the century (~1000-1100 µatm CO_2) are compared to current, ambient conditions throughout the thesis.

In order to further explore the effects on the survivors the second manuscript ("Impacts and Trade-offs of Ocean Acidification on Growth, Skeletal, and Organ Development of Atlantic Cod Larvae") investigates the impact on growth and development on the Arcto-Norwegian cod larvae. It examines the trade-offs due to food availability that the larvae have to make while coping with the stressor of hypercapnia. Changes in skeletal ossification and organ development, particularly of the gills, the liver and the eyes, are described.

The third manuscript on "Parental acclimation effects in response to ocean acidification in Atlantic cod" investigates whether the larval response is changed by parental exposure to acidification. Survival is measured in response to larval and parental exposure to high CO_2 as well as in response to food availability. Organ impairments due to acidification is also explored.

1.7.1 List of Manuscripts for Thesis

The chapters of this doctoral thesis are based on the following manuscripts:

I Stiasny, M.H., Mittermayer, F.H., Sswat, M., Voss, R., Jutfelt, F., Chierici, M., Puvanendran, V., Mortensen, A., Reusch, T.B.H., Clemmesen, C. (2016) **Ocean Acidification Effects on Atlantic Cod Larval Survival and Recruitment to the Fished Population**, PLoS ONE, 11 (8)

II Stiasny, M.H., Sswat, M., Mittermayer, F.H., Falk-Petersen, I.-B., Schnell, N.K., Puvanendran, V., Mortensen, A., Reusch, T.B.H., Clemmesen, C., **Impacts and Trade-offs of Ocean Acidification on Growth, Skeletal, and Organ Development of Atlantic Cod Larvae**, to be submitted to Global Change Biology

III Stiasny, M.H., Mittermayer, F.H., Göttler, G., Bridges, C. R., Falk-Petersen, I-B., Puvanendran, V., Mortensen, A., Reusch, T.B.H., Clemmesen, C., **Parental Acclimation Effects in Response to Ocean Acidification in Atlantic Cod**, submitted to Scientific Reports

1.8 Declaration of Contribution

Manuscript I

Idea: **Martina H. Stiasny**, Catriona Clemmesen, Michael Sswat

Data acquisition:

Martina H. Stiasny, Michael Sswat, Fredrik Jutfelt, Felix H. Mittermayer and Catriona Clemmesen performed the experiments; Velmurugu Puvanendran and Atle Mortensen supported the experiment in Tromsø; Melissa Chierici performed the carbonate chemistry analysis;

Data interpretation and manuscript preparation:

Rüdiger Voss included the experimental survival data into the recruitment analysis and wrote the section on that topic;

Martina H. Stiasny analyzed the data and wrote the main paper, including all figures, tables and statistical analyses with support from Catriona Clemmesen and Thorsten Reusch

Manuscript II

Idea: **Martina H. Stiasny**, Catriona Clemmesen, Michael Sswat

Data acquisition:

Martina H. Stiasny, Michael Sswat, Felix H. Mittermayer and Catriona Clemmesen performed the experiments; Velmurugu Puvanendran and Atle Mortensen supported the experiment, Nalani K. Schnell performed the ossification analysis, Inger-Britt Falk-Petersen performed the histological analysis

Data interpretation and manuscript preparation:

Martina H. Stiasny analyzed the data and wrote the main paper, including all figures, tables and statistical analyses with support from Catriona Clemmesen and Thorsten Reusch

Manuscript III

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Data acquisition:

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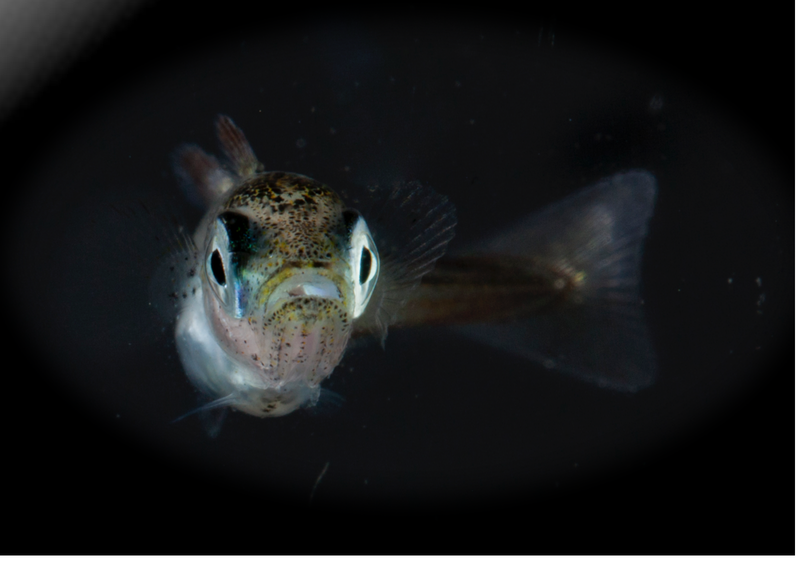
Data interpretation and manuscript preparation:

Martina H. Stiasny analyzed the data and wrote the main paper, including all figures, tables and statistical analyses with support from Catriona Clemmesen and Thorsten Reusch

2 MANUSCRIPT I

Ocean Acidification Effects on Atlantic Cod Larval Survival and Recruitment to the Fished Population



Ocean Acidification Effects on Atlantic Cod Larval Survival and Recruitment to the Fished Population

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Published: 23rd August 2016

doi:10.1371/journal.pone.0155448

all data files available from the pangaea database under https://doi.pangaea.de/10.1594/PANGAEA.858616.

2.1 Abstract

How fisheries will be impacted by climate change is far from understood. While some fish populations may be able to escape global warming via range shifts, they cannot escape ocean acidification (OA), an inevitable consequence of the dissolution of anthropogenic carbon dioxide (CO_2) emissions in marine waters. How ocean acidification affects population dynamics of commercially important fish species is critical for adapting management practices of exploited fish populations. Ocean acidification has been shown to impair fish larvae's sensory abilities, affect the morphology of otoliths, cause tissue damage and cause behavioural changes. Here, we obtain first experimental mortality estimates for Atlantic cod larvae under OA and incorporate these effects into recruitment models. End-of-century levels of ocean acidification (~1100 µatm according to the IPCC RCP 8.5) resulted in a doubling of daily mortality rates compared to present-day CO_2 concentrations during the first 25 days post hatching (dph), a critical phase for population recruitment. These results were consistent under different feeding regimes, stocking densities and in two cod populations (Western Baltic and Barents Sea stock). When mortality data were included into Ricker-type stock-recruitment models, recruitment was reduced to an average of 8 and 24% of current recruitment for the two populations respectively. Our results highlight the importance of including vulnerable early life stages when addressing effects of climate change on fish stocks.

2.2 Introduction

The understanding of the effect of global change on fish populations is critical for sustainable exploitation and management of fisheries (MacNeil *et al.*, 2010). Ocean warming has already triggered poleward range shifts of many marine fish populations caused by their thermal tolerance (Perry *et al.*, 2005; Pörtner, 2010; Poloczanska *et al.*, 2013). However, higher latitudes provide no refuge with respect to the concomitant pH decline, caused by the dissolution of the major greenhouse gas CO_2 in ocean waters. This “other CO_2 problem”, also dubbed ocean acidification (OA) (Doney *et al.*, 2009), is an inevitable consequence of anthropogenic release of CO_2. The potential consequences of ocean acidification on commercially important fish populations are intensely debated (Baumann *et al.*, 2012; Lam *et al.*, 2014), but currently unresolved since data on population-level processes, e.g. recruitment to the stock, are almost entirely lacking (Denman *et al.*, 2011; Cheung *et al.*, 2012; Haigh *et al.*, 2015).

Adult fishes have been shown to tolerate extreme CO_2 concentrations of up to 16,000 µatm (Ishimatsu *et al.*, 2008), which led to the premature conclusion that fishes are less vulnerable to ocean acidification than for example calcifying organisms (Kroeker *et al.*, 2013). However, it is becoming increasingly evident that early life stages such as eggs and larvae are more susceptible to decreased ocean pH (7,13). This is partly due to insufficient acid-base regulation prior to the formation of gills (Falk-Petersen, 2005). Recent studies have shown a diverse range of impacts of predicted future CO_2 concentrations on larval fish, particularly on sensory abilities like olfaction (Munday *et al.*, 2009a), behaviour (Munday *et al.*, 2010; Dixson *et al.*, 2012), otoliths (Checkley *et al.*, 2009; Bignami *et al.*, 2013; Maneja *et al.*, 2013), development, tissue and organ structure (Frommel *et al.*, 2012, 2014). Studies also found effects on survival of eggs, more specifically hatching success (Chambers *et al.*, 2013), and survival of very early larval stages (Baumann *et al.*, 2012; Bromhead *et al.*, 2015). Other studies were not able to find an effect on survival (Munday *et al.*, 2009b, 2015).

Survival, however, is the most important parameter to assess recruitment, thus of paramount importance for stock management. Recruitment to an exploited fish stock is defined as that point of time when a year-class enters the fished population, i.e. at an age of 1 year in the case of Western Baltic cod, and at an age of 3 years in Barents Sea cod. Here we assess larval mortality as a key variable to predict population growth and size (Houde, 2008; Llopiz *et al.*, 2014) in Atlantic cod (*Gadus morhua,* L.) under end-of-century CO_2 concentrations. This is one of the most important species for commercial fisheries of the North Atlantic,. It is of particular importance since landings of many cod stocks have decreased in the past decades with some stocks collapsing (Pauly *et al.*, 2002). Any additional source of mortality, particularly one with a trend, should therefore be closely monitored and incorporated into management strategies.

We designed two experiments, in which the survival of cod larvae was quantified in direct response to increased pCO_2 levels as predicted for the end of the century. Atmospheric CO_2 concentrations have been continuously rising since the beginning of industrialisation and are currently exceeding 400 µatm. A third of the excess CO_2 is absorbed by the world’s oceans, resulting in ocean acidification, leading to an estimated decrease in pH of 0.4 units (pCO$_2$ ~ 1,000 µatm) by the end of the century (5,29,30). Eggs and larvae from the Western Baltic cod stock, caught in the Øresund, and from the Arcto-Norwegian Barents Sea cod stock were kept under control (~400-500 µatm) and high CO$_2$ (~1100 µatm) concentrations in two separate experiments until 25 and 22 days post-hatching (dph) respectively and survival was monitored closely.

2.3 Methods and Materials

For the Western Baltic experiment, adult cod were caught in the Øresund (55°58'N, 12°38'E) in March 2013 and strip-spawned. An equal volume of eggs was placed in 90 L rearing tanks at the Sven Lovén Centre, Kristineberg, Sweden. Three tanks were kept under ambient CO$_2$ concentrations of 426 ± 47 µatm and three tanks were kept under increased CO$_2$ conditions of 1033 ± 255 µatm. The temperature was kept constant at 7°C and the light regime was matched weekly to the ambient sun rise and sun set. After hatching the larvae were fed with natural plankton from the Gullmars Fjord under green water conditions with *Nannochloropsis*. (Food density estimates are given in Supporting Information SI Table 2.1). Survival was measured daily by collecting and counting all dead larvae from the bottom of the tanks. Initial number of larvae (on average ~800 larvae per tank) was then back-calculated to calculate survival in percentage. It was shown in separate experiments that dead larvae were easily found even after more than 24 hours post mortem in the tanks.

For the Barents Sea cod experiment adult fish were caught alive in the Barents Sea (70°15'N, 19°00'E) in March 2014 and transferred to the National Cod Breeding Centre, Tromsø. They were kept in large breeding tanks (25 m^3) with flow-through from the fjord and at weekly matched ambient light regimes. All naturally produced eggs were collected using collectors behind the surface skimmer outflow. These were transferred to incubators with either ambient (503 ± 89 µatm CO$_2$) or increased CO$_2$ (1179 ± 87 µatm) concentrations. After peak hatch (more than 50% eggs hatched), 11,000 larvae were transferred into each of twelve 190 L rearing tanks with a constant flow-through of water from a common header tank. For the egg incubation and the start of the experiment the temperature was set to 6°C and was later raised to 10°C in all tanks at constant light conditions (24h). Larvae were fed with *Nannochloropsis* and *Brachionus* at different intervals for the high and the low food treatment (seven compared to three times daily), while the prey concentrations per feeding remained the same for both treatments. (For information on the feeding conditions, see Supporting Information SI Table 2.2). Larvae in one tank in the ambient CO$_2$ treatment were abruptly lost over night, due to an unknown factor, resulting in six replicates for the high CO$_2$ treatment and five for the ambient treatment, each divided equally into the high and low food treatment. Starting on 8 dph survival was measured every four to six days by calculating the density of the larvae in the tanks. Five times 0.8 l of water was sampled from each tank over the whole water column using a pipe that could be closed at the bottom and the larvae contained in the pipe were subsequently counted in each sub sample. Prior to sampling an even distribution of larvae in the rearing tanks was achieved by increasing the aeration.

For both experiments the mean mortality coefficient was calculated after non-linear curve fitting of a negative exponential function for each replicate tank. Mean daily mortality rates (in percentage per day) were compared between treatments using a t-test (Western Baltic stock) and a two-way ANOVA (Barents Sea stock) after appropriate data transformation to achieve homogeneity of variances.

Ambient and increased CO_2 levels were achieved by controlling the pH values in a header tank with pH sensors connected to an IKS computer system. If the values deviated from the set target pH a magnetic valve opened automatically, which allowed a pulse of CO_2 from a CO_2 bottle to be injected into the header tank. The volume of the header tank ensured a thorough mixing and equilibration of CO_2 before the water entered the rearing tank thereby assuring constant conditions in the rearing tanks. The pH was furthermore manually checked every day in the rearing tanks with a separate pH sensor (WTW pH/Cond 340i/3320). Water chemistry, including DIC and alkalinity, was tested at the beginning and the end of the experiment for the Western Baltic cod experiment and weekly for the Barents Sea cod experiment based on the Best Practices Guide (Riebesell *et al.*, 2010). Further details regarding methods and carbon chemistry analysis are available in the Supporting Information.

All experiments were carried out in accordance to the national rules and regulations at the site of the experiments and all efforts where undertaken to minimize stress and suffering of the animals. Issues for work on vertebrate animals were obtained for each experiment and location. For the experiment in Kristineberg with the Western Baltic cod the ethics permit number is 332-2012 issued by the Swedish Board of Agriculture (Jordbruksverket). For the experiment in Tromsø on the Barents Sea cod the ethics permit number is FOTS ID 6382, issued by the Norwegian Animal Research Authority (Forsøksdyrutvalget). In accordance with these permits animals were euthanized after the experiment or whenever some were taken out for density measurements using Tricaine methanesulfonate (MS222). No endangered or protected species were used in these experiments and no other special permits for necessary.

Population level effects

Considering the potential impact of ocean acidification on fisheries requires scaling from physiological responses to population-level processes. A simple way is to consider how ocean acidification could modify the parameters of growth, mortality and reproduction in a single-species. Here we concentrate on the modification of the parameters of the stock-recruitment relationship in an age-structured fishery model.

The effect of ocean acidification was assessed by modifying the density-independent parameter a of a Ricker type stock recruitment relationship. Ocean acidification causes a higher larval mortality rate. This leads to a density-independent mortality rate a caused by acidification. In the baseline scenario (no acidification) $a=0$, while in the acidification scenarios, e^{-a} is the fraction of larvae surviving the effect of acidification. We used our experimental data to quantify this effect, and to compare scenarios (See Supporting Information). We used ICES data for Western Baltic cod for the years 1970 to 2014 and for Arcto-Norwegian cod for the years 1946-2014 to estimate the stock-recruitment relationship for the baseline scenario. We assume log-normal auto-correlated errors, and estimated the model. (Further details regarding the recruitment models are available as Supporting Information.) Because the severity of ocean acidification induced mortality on recruitment depends on the duration of the additional mortality, two developmental stages were chosen as termination for the enhanced mortality. Based on the experimental temperatures at day 23 days post hatching the larval gut has reached its typical spiral form (and potentially altered function) while at 30 dph gills become visible on the gill arches. These two time points were used to evaluate the effect of increased mortality on recruitment success assuming the same mortality estimates until 30 dph as shown in the experiments until 22 dph and 25 dph. Mortality during the recruitment process consists of both density-independent and density-dependent effects. For simplicity we assume that the effect of ocean acidification on the survival will only influence the density-independent mortality during the recruitment phase potentially biasing the data to be on the conservative side.

2.4 Results

The effect of CO_2 was consistent among stocks and experimental conditions, i.e. different feeding conditions. At increased CO_2 concentrations the daily mortality rates had approximately doubled in both experiments, from 7 to 13% in the Barents Sea stock (Figure 2.1a) and from 9.2 to 20.4% in the Western Baltic Sea stock (Figure 2.1b) (Western Baltic experiment, T-test, t=-3.749, df=2.41, p=0.024; Barents Sea experiment Two-way ANOVA F=8.434, df= 1, p=0.023). In the Barents Sea experiment the food density had no detectable effect on mortality rate, neither as main effect nor in interaction with the CO_2-treatment (for additional statistics, see Supporting Information SI Tables 2.3 and 2.4). Cod larvae therefore appear to be negatively affected by ocean acidification even when *ad libitum* prey densities should ensure that energy is available for potential acid-base regulation mechanisms.

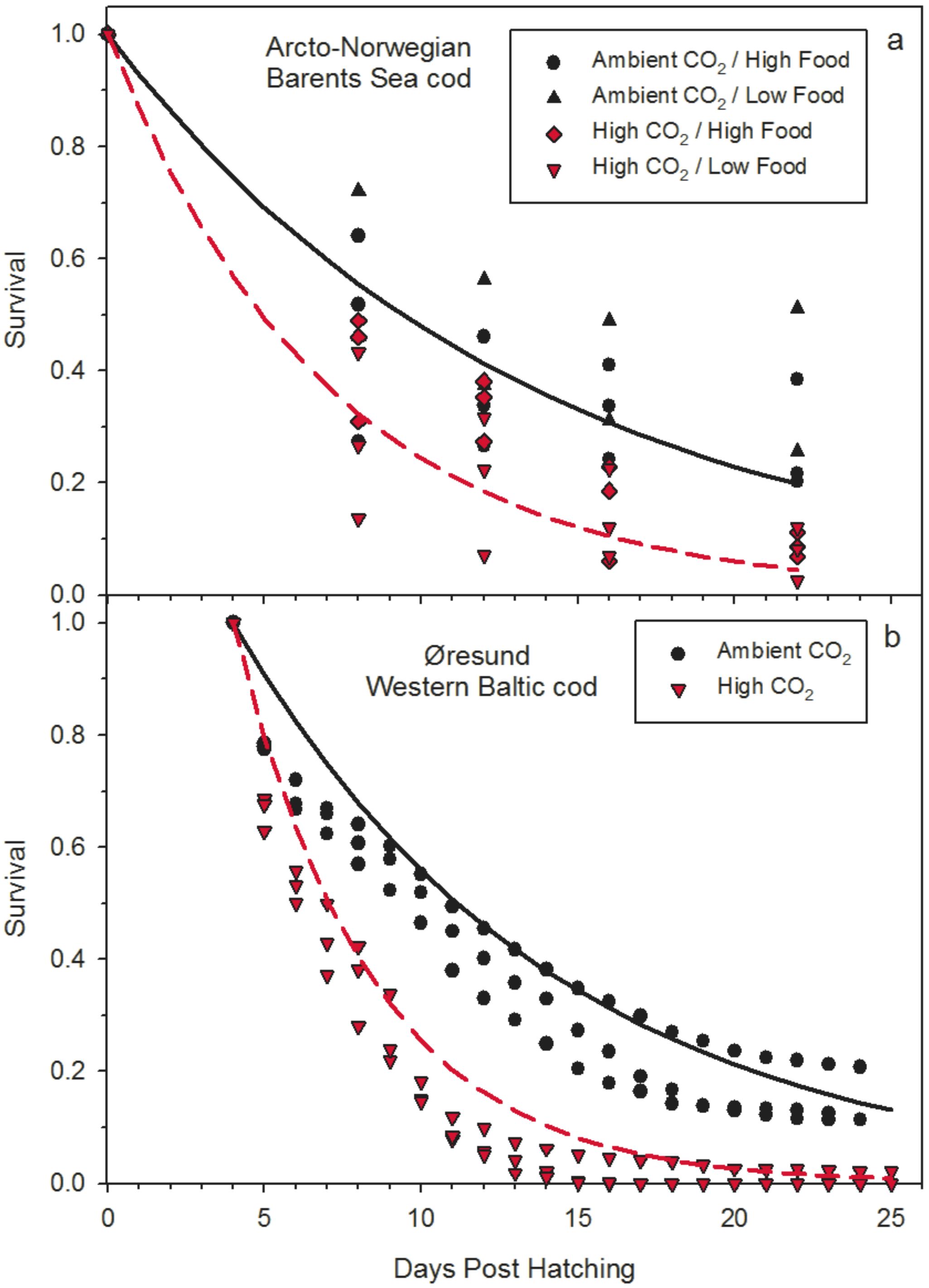


Figure 2.1. Effect of increased CO_2 on early life survival of *Gadus morhua* from a) Barents Sea cod b) Western Baltic cod. Each symbol represents the value of one replicate tank. Lines depict the number of survivors according to the fitted negative exponential function.

Next, the experimentally assessed larval mortality rates were incorporated into a Ricker-type stock-recruitment model that was parameterized for the two studied cod populations. We concentrated on altering the larval mortality in order to evaluate the overall stock-recruitment relationship to assess their effects on population dynamics (for details see Supporting Information). The model results show that for both mortality scenarios increased larval mortality due to ocean acidification will reduce recruitment substantially. Recruitment levels will be reduced on average to only 8% of the baseline scenario in the case of Western Baltic cod for ocean acidification-induced mortality periods of 23 days (and 4% for a mortality period of 30 days), and to 24.5% (and 17% respectively) in Arcto-Norwegian cod (Figures 2.2 and 2.3).

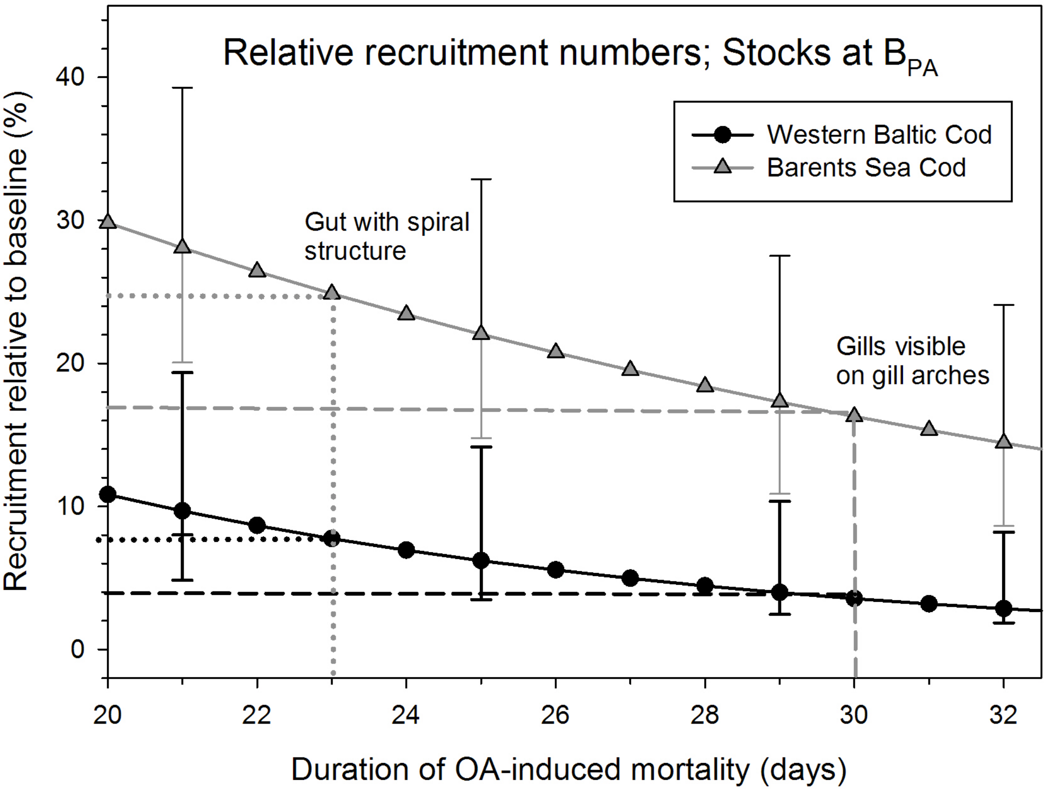


Figure 2.2. Recruitment functions under baseline and under ocean acidification scenarios for (a) the Barents Sea cod and (b) the Baltic Sea cod. The baseline scenario is based on no OA and spawning stock biomass at ICES precautionary biomass levels (B_{PA}) in dependence of the duration of OA-induced mortality. For better visualization is the recruitment under OA on the second y-axes with different ranges.

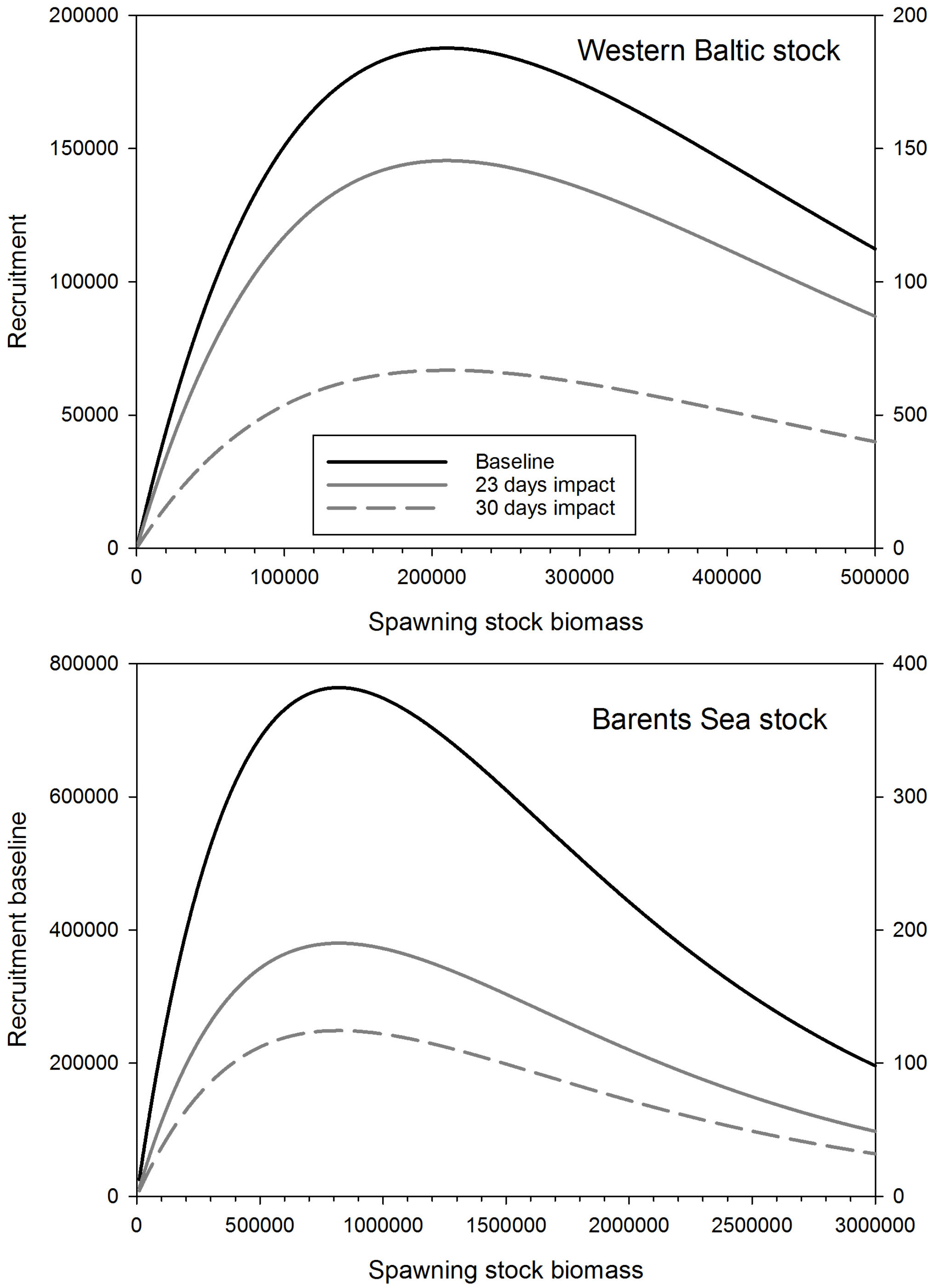


Figure 2.3. Population recruitment under ocean acidification (OA) for Western Baltic cod (black line and symbols) and Barents Sea cod (grey line and symbols). Recruitment is given relative to a baseline scenario of no OA and spawning stock biomass at ICES precautionary biomass levels (B_{PA}) in dependence of the duration of OA-induced mortality. Two important points in larval development are highlighted. Standard deviations displayed only for selected days to improve readability.

2.5 Discussion

Under realistic scenarios of end-of-century ocean acidification, early larval survival of cod was significantly reduced in two separate experiments with two different Atlantic cod stocks. Results were consistent under different feeding regimes and strongly suggest that there is a severe effect of ocean acidification on Atlantic cod larvae and recruitment.

Mass spawning fishes such as cod have many offspring with low survival probability in nature. The salient question is whether our experimental conditions provide appropriate controls with reasonable natural mortality levels. Larval survival rates are naturally low even under ambient CO_2 concentrations and optimal feeding conditions. The mortality is mainly caused by the difficulty in a successful first feeding once the yolk sac is absorbed (Houde, 2008). Other studies find similar mortality rates as our control values in the two experiments during early larval development (Puvanendran & Brown, 1999; van der Meeren *et al.*, 2007). Survival of larvae in our experiment from the Western Baltic stock was lower than for the Barents Sea stock, since they were fed with natural plankton in concentrations as provided by the fjord, while the larvae from the Barents Sea stock were kept under aquaculture conditions aiming for the production of the highest numbers of fingerlings for stocking of industrial scale production net pens.

Larval fish survival under ocean acidification has so far been shown in only one other study by Baumann *et al.* (2012), albeit in a non-commercial fish species, the Atlantic silverside (*Menidia menidia*). In their study reduced larval survival was observed at 1100 *ppm* during the first week post hatch, a level of ocean acidification, which is predicted to occur globally at the start of the next century under the IPCC RCP 8.5. Chambers *et al.* (2013) found a decreased hatching success (reflecting embryonic development) of the summer flounder by 50% under 1860 *ppm*, a realistic ocean acidification level for the environment of this species within this century, even though values on a global average are predicted to be lower. Munday *et al.* (2015) found no effect on the survival of yellowtail kingfish larvae. Other studies, like Munday *et al.* (2009b); Franke & Clemmesen (2011); Frommel *et al.* (2013); Hurst *et al.* (2013, 2015), have addressed hatching success and have not seen any effects of ocean acidification. We are confident that this does not necessarily indicate that these species will not be affected or that our results present a contradiction. It is well known that early life stages of marine fish go through several bottlenecks with high mortalities during development and that different populations of the same species can react differently to CO_2 stress (Frommel *et al.*, 2013). Our results show that the first days and weeks after hatching are a vulnerable phase to ocean acidification. So far studies on tropical fish have not seen an ocean acidification effect on survival (Munday *et al.*, 2011). This is not surprising, since early development in the studied species is very different from temperate fish and newly hatched larvae are further developed and physiologically more competent thus less vulnerable to physiological stressors. Furthermore the study by Munday *et al.* (2011), and other studies like Hurst *et al.* (2013), only quantified survival at a single day, which may not have been the final day of any additional mortality. Additionally, even if this was an end-point measurement, it does not allow for calculations of mortality rates.

One factor that this study is not taking into account is possibility that parental exposure to the high CO_2 environment could limit the adverse effects of ocean acidification. This kind of transgenerational adaptation has been shown to mediate negative growth effects of OA in tropical reef fish (Miller *et al.*, 2012). However since most commercially important fish species are quite large and temperate fish species reach sexual maturity late, it will be difficult to perform experiments with long parental exposure time. Furthermore it cannot be ruled out, that ocean acidification might also have an additional negative effect on gonadal development in adult fishes, which might further reduce recruitment potential.

Range shifts are responses of many fish populations to track the poleward movement of their thermal range (Perry *et al.*, 2005). Unfortunately, this may exacerbate direct CO_2 effects identified here, since oceanic waters in higher latitudes will take up more CO_2 due to higher solubility and experience lower carbonate saturation (Orr *et al.*, 2005). Previously, ocean acidification has been shown to affect marine fish larvae's sensory abilities, morphology of the otoliths, cause tissue damage and behavioural differences (Checkley *et al.*, 2009; Munday *et al.*, 2010; Frommel *et al.*, 2012, 2014; Bignami *et al.*, 2013).

Here we give the first demographic estimates for Atlantic cod under realistic end-of-century ocean acidification levels which are urgently needed to estimate whether these exploited fish populations could potentially expect population declines as a direct consequence of ocean acidification. The estimated recruitment declines shown are severe, of similar magnitude as population collapses due to overfishing (Pinsky *et al.*, 2011) and have highly significant implications for the governance of exploited fish populations. We show that indeed, increased mortality will affect recruitment at the population level, demonstrating that any future management of exploitation must directly consider effects induced by global change.

2.6 Acknowledgements

Funding was provided through the BIOACID project (BIOlogical Impacts of Ocean ACIDification) funded by the German Ministry for Education and Research (BMBF), the AquaExcel transnational access grant for aquaculture infrastructures and the Bonus Baltic Sea research and development programme (Art 185) BIO-C3 project, funded jointly by the EU and the BMBF (Grant No. 03F0682A). The Western Baltic cod experiment was conducted at the Sven Lovén Centre, Kristineberg, Sweden. The Barents Sea cod experiment was conducted at the Norwegian Cod Breeding Centre, NOFIMA, Tromsø, Norway. The authors are grateful to all scientists and staff at both research stations.

2.7 Supporting Information

Experimental set-up

For the Western Baltic experiment, adult cod were caught in the Øresund (55°58'N, 12°38'E) in March 2013 and strip-spawned to create fifteen families (3 females x 5 males). An equal volume of eggs was placed in 90 L rearing tanks at the

Sven Lovén Centre, Kristineberg, Sweden. Three tanks were kept under ambient CO_2 concentrations of 426 ± 47 µatm and three tanks were kept under increased CO_2 conditions of 1033 ± 255 µatm. The temperature was kept constant at 7°C and the light regime was matched weekly to the ambient sun rise and sun set. After hatching the larvae were fed with natural plankton from the Gullmars Fjord (daily concentrations are shown in SI Table 2.1) and with *Nannochloropsis*.

SI Table 2.1. Feeding densities for the Western Baltic stock.

	Kristineberg Natural Plankton			
dph	*Nannochloropsis* added	First Daily Feeding (prey org ml^{-1} $feeding^{-1}$)	Second Daily Feeding (prey org ml^{-1} $feeding^{-1}$)	Third Daily Feeding (prey org ml^{-1} $feeding^{-1}$)
1	yes	0	0	0
2	yes	0	0	0
3	yes	0	0	0
4	yes	0	0	0
5	yes	0.18	0	0
6	yes	0	0	0
7	yes	0.34	0	0
8	yes	0.51	0	0
9	yes	0.20	0.18	0
10	yes	0.18	0.34	0
11	yes	0.70	0	0
12	yes	0.70	0.67	0
13	yes	0.37	0.34	0
14	yes	0.43	0.48	0
15	yes	0.65	0.55	0.44
16	yes	0.18	0.40	0
17	yes	0.12	0.17	0.20
18	yes	0.24	0.34	0
19	yes	0.13	0.19	0
20	yes	0.28	0.15	0.18
21	yes	0.11	0.08	0
22	yes	0.17	0.12	0.15
23	yes	0.21	0.08	0
24	yes	0.12	0	0
25	yes	0.27	0.86	0

Survival was measured daily by collecting all dead larvae from the bottom of the tanks and counting these. Initial number of larvae was then back-calculated to calculate survival in percentage. It was shown in separate experiments that dead larvae were easily found even after more than 24 hours in the tanks.

For the Barents Sea cod experiment adult fish were caught alive in the Barents Sea (70°15'N, 19°00'E) in March 2014 and transferred to the National Cod Breeding Centre, Tromsø. They were kept in large breeding tanks (25 m^3) and all produced eggs were collected from the outflow. These were transferred to incubators with either ambient (503 ± 89 µatm) or increased CO_2 (1179 ± 87 µatm) concentrations. After peak hatch (more than 50% eggs hatched), 11,000 larvae were transferred into each of twelve 190 L rearing tanks with a constant flow-through of water from a common header tank. For the egg incubation and the start of the experiment the temperature was set to 6°C and was later raised to 10°C in all tanks at constant light conditions (24h). Larvae were fed with enriched rotifers. Densities and number of daily feedings can be found in SI Table 2.2.

SI Table 2.2. Feeding densities for the Barents Sea stock.

	Tromsø Low Food			Tromsø High Food		
dph	*Nannochloropsis* added	prey org ml^{-1} $feeding^{-1}$	number of daily feedings	*Nannochloropsis* added	prey org ml^{-1} $feeding^{-1}$	number of daily feedings
1	yes	3.2	7	yes	3.2	7
2	yes	3.2	7	yes	3.2	7
3	yes	3.2	7	yes	3.2	7
4	yes	3.2	7	yes	5.5	7
5	yes	5.5	7	yes	5.5	7
6	yes	5.5	7	yes	5.5	7
7	yes	5.5	7	yes	5.5	7
8	yes	5.5	7	yes	5.5	7
9	yes	5.5	7	yes	5.5	7
10	yes	5.5	7	yes	5.5	7
11	yes	5.5	7	yes	5.5	7
12	yes	5.5	7	yes	5.5	7
13		5.5	7		5.5	7
14		5.5	3		5.5	7
15		5.5	3		5.5	7
16		5.5	3		5.5	7
17		5.5	3		5.5	7
18		5.5	3		5.5	7
19		5.5	3		5.5	7
20		5.5	3		5.5	7
21		5.5	3		5.5	7
22		5.5	3		5.5	7

Larvae in one tank in the ambient CO_2 treatment were abruptly lost over night, due to an unknown factor, resulting in six replicates for the high CO_2 treatment and five for the ambient treatment. Starting on 8 dph the survival was measured every four to six days by calculating the density of the larvae in the tanks, sampling five times 0.8 L of water from the tanks over the whole water column using a pipe that could be closed at the bottom and then counting larvae in this sub sample. An even distribution was achieved by increasing the air inflow through the aeration stones.

Set-up and determination of the CO_2-system

Ambient and high CO_2 levels were achieved by controlling the pH values in a header tank with pH probes connected to a computer monitoring system (IKS-aquastar). If the values deviated from the set target pH a magnetic valve was opened, which allowed a pulse of CO_2 from a CO_2 bottle to go into the header tank. The volume of the header tank ensured a thorough mixing and equilibration of CO_2 before the water entered the rearing tank thereby assuring constant conditions in the rearing tanks. The pH was furthermore manually checked every day in the rearing tanks with a separate pH probe (WTW pH/Cond 340i/3320). Water chemistry, including C_T (total carbon) and A_T (total alkalinity), was tested at the beginning and the end of the experiment for the Western Baltic cod experiment and weekly for the Barents Sea cod experiment based on the Best Practices Guide (Riebesell *et al.*, 2010).

Analytical methods for C_T (total carbon) and A_T (total alkalinity) determination in seawater samples are fully described in Dickson *et al.* (2007). Briefly, C_T was determined using gas extraction of acidified sample followed by coulometric titration and photometric detection using a Versatile Instrument for the Determination of Titration carbonate (VINDTA 3C, Marianda, Germany). A_T was determined in water column samples from potentiometric titration with 0.1 N hydrochloric acid using a Versatile Instrument for the Determination of Titration Alkalinity (VINDTA 3C, Marianda). The average standard deviation for C_T and A_T, determined from replicate sample analyses from one sample, was within ±1 μmol kg^{-1}. The accuracy of the measurements were ensured by routine analyses of Certified Reference Materials (CRM, provided by A. G. Dickson, Scripps Institution of Oceanography, USA) and was better than ±1 μmol kg^{-1} and ±2 μmol kg^{-1} for C_T and A_T, respectively.

We used C_T, A_T, salinity, and temperature, for each sample as input parameters in a CO_2-chemical speciation model (CO2SYS program (Pierrot *et al.*, 2006)) to calculate all the other parameters in the CO_2-system such as pH in situ, CO_2 fugacity and partial pressure ($f\mathrm{CO_2}$, $p\mathrm{CO_2}$), carbon dioxide concentration ($[CO_2]$) and carbonate-ion concentration ($[CO_3^{2-}]$), and calcium-carbonate saturation states in the water column (Ω) for aragonite (Ω_{Ar}) and calcite (Ω_{Ca}), We used the total hydrogen-ion scale (pH_T), the HSO_4^- dissociation constant of Dickson (1990) and the CO_2-system dissociation constants (K^*_1 and K^*_2) estimated by Mehrbach *et al.* (1973) refit by Dickson & Millero (1987). Mean values and standard deviation of $p\mathrm{CO_2}$ in the Western Baltic cod experiment were 1033 +- 255 µatm for the high and 426 +- 47 µatm for the ambient treatment which is equivalent to a pH value (total scale at in situ temperature) of 7.76 +- 0.09 for the high and 8.17 +- 0.03 at ambient conditions. Mean values and standard deviation of $p\mathrm{CO_2}$ in the Barents Sea cod experiment were 1179 +- 87 µatm for the high and 503 +- 89 µatm for the ambient treatment which is equivalent to a pH value (total scale at in situ temperature) of 7.61 +- 0.03 for the high and 7.90 +- 0.15 at ambient conditions.

Statistics

Data were cubic-root transformed to achieve variance homogeneity, assessed with Bartlett's test. Results are shown in SI 5.3 and SI 5.4.

SI 5.3. Statistics for the Western Baltic cod stock.

Source of variation	Degrees of freedom	t-ratio	p-value
CO_2	2.41	-3.749	0.024

SI 5.4. Statistics for the Barents Sea cod stock.

Source of variation	Degrees of freedom	F	p-value
CO_2	1	8.434	0.023
Food	1	0.06	0.814
CO_2*Food	1	2.325	0.171

Recruitment model

Experimental studies, like the one presented here, mostly refer to effects of ocean acidification on physiological processes. Considering the potential impact of ocean acidification on fisheries requires scaling from physiological responses to population- and ecosystem-level processes. A simple way is to consider how ocean acidification could modify the parameters of growth, mortality and reproduction in a single-species model(Le Quesne & Pinnegar, 2012). Here we concentrate on the modification of the parameters of the stock-recruitment relationship in an age-structured fishery model. For visualization purposes we choose recruitment at the management target of precautionary biomass levels (B_{PA}) as given by ICES (ICES, 2014a, 2014b).

We assume that egg production in N_0, is proportional to spawning stock biomass, SSB, i.e. $N_0 = f\mathrm{SSB}$, where *f* is the net fecundity in the population (Hilborn & Walters, 1992). We assume that the stock-recruitment relationship is of the Ricker(Ricker, 1954) type. Such a type of stock-recruitment relationship is an appropriate description of recruitment biology of cod (Cook *et al.*, 1997). According to the Ricker model (Ricker, 1954; Quinn & Deriso, 1999), the development of the early-life history follows $dN(\tau)/d\tau = -\frac{(a+b+\phi_2\, SSB)}{T}N(\tau)$, where $N(0) = N_0$, and recruits enter the fish stock at $T = 1,3$ years, respectively, depending on the fish stock. Natural mortality $\frac{a+b+\phi_2\, SSB}{T}$ is made up of three components. Ocean acidification causes a higher larval mortality rate. This leads to a density-independent mortality rate *a/T* caused by acidification. Furthermore, *b*/T is the density-independent mortality rate at baseline conditions, and $\frac{\phi_2 \, SSB}{T}$ is the density-dependent which increases with the spawning stock, e.g. because of cannibalism. Solving the differential equation, we obtain

$$R = f \, SSB e^{-a-b-\phi_2 SSB} = e^{-a} \phi_1 \, SSB \, e^{-\phi_2 SSB}$$

Where R denotes recruits in numbers, and $\phi_1 = f e^{-b}$. In the baseline-scenario, we have $a = 0$, in the acidification scenarios, e^{-a} is the fraction of cod in the early life history stages that survives the effect of acidification. We use the data from experiments to quantify this effect.

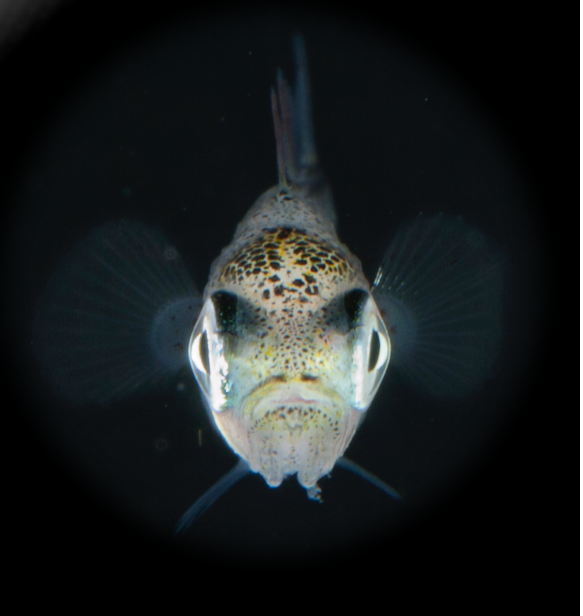
To estimate the stock-recruitment relationship for the baseline scenario we use ICES data for Western Baltic cod for the years 1970 to 2014 and for the Barents Sea cod for the years 1946-2014. We assume log-normal auto-correlated errors, and estimate the model

$$\ln(R) = \ln(\phi_1 SSB) - \phi_2 SSB + \xi_t,$$

where $\xi_{t+1} = v\xi_t + \varepsilon_t$, and ε_t is a series of iid random variables. We obtain estimates $\ln(\phi_1)$ = 0.929 with 95% confidence interval [1.05; 0.808] and ϕ_2 = -1.219/million tons with 95% confidence interval [-0.999; -1.439]/million tons for the Barents Sea cod as well as $\ln(\phi_1)$ = 0.888 with 95% confidence interval [1.224; 0.553] and ϕ_2 = 4.762/million tons with 95% confidence interval [-4.672; 14.196]/million tons for Western Baltic cod.

3 MANUSCRIPT II

Impacts and Trade-offs of Ocean Acidification on Growth, Skeletal, and Organ Development of Atlantic Cod Larvae



Impacts and Trade-offs of Ocean Acidification on Growth, Skeletal, and Organ Development of Atlantic Cod Larvae

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In preparation for submission to Global Change Biology

3.1 Abstract

In order to understand the effect of global change on marine fish populations, it is imperative to quantify the effects on fundamental parameters such as survival and growth. It has been previously shown that larval survival and recruitment of the Arcto-Norwegian cod may be heavily impaired by realistic, end-of-century levels of ocean acidification. Here, we show that larval growth among the surviving larvae is affected, along with organ development and the ossification of the skeleton. We also manipulated food availability in order to evaluate the effect of energy limitation on acidification effects. Acidification showed a significant interaction with food treatment with size and skeletogenesis being generally positively affected by increased food availability. Larvae fed ad libitum showed little difference in growth and skeletogenesis between the ambient and high CO_2 treatment, while larvae, which were energy limited, were significantly larger and had further developed skeletal structures in the acidified treatment versus the ambient CO_2 treatment. However, the latter group revealed impairments in certain organs, such as the liver, and had comparatively smaller functional gills. It is therefore likely that individual larvae that had survived acidification treatments, will suffer from impairments later during ontogeny. This may be due to an allocation trade-off, which the larvae have to make between growth and other more specific developmental processes.

3.2 Introduction

Fish populations are progressively subjected to global change, for example through increasing temperatures and ocean acidification. A thorough understanding of these effects on individuals and populations therefore becomes increasingly important, particularly for species that are commercially exploited and therefore need to be managed accordingly. Studies on the effects of ocean acidification on larval fish growth have shown pronounced interspecies variability. For example, no effect on growth rate was observed for walleye Pollack (Hurst *et al.*, 2013), whereas a decrease in growth rate under high $p\text{CO}_2$ was observed in Atlantic silverside (Baumann *et al.*, 2012) and summer flounder (Chambers *et al.*, 2013) compared to an increase in growth rate under high $p\text{CO}_2$ levels in cod (Frommel *et al.*, 2012) and sand smelt larvae (Silva *et al.*, 2016).

No generalizations are currently possible among species, possibly even among stocks of the same species. Generally it is believed that enhanced growth leads to higher survival in eaerly-life-stages, due to less predation, since larvae outgrow the predator field faster (Bailey & Houde, 1989). Therefore there is evolutionary pressure for fast growth (Houde, 1997). This has been shown for certain Atlantic cod stocks (Meekan & Fortier, 1996). It is therefore increasingly important to understand the underlying mechanisms of ocean acidification leading to the effect on size and how trade-offs, made by the larvae, may manifest in changed growth patterns.

The Atlantic cod (*Gadus morhua*) is one of the most important commercial species and the Arcto-Norwegian cod stock in particular supports a large fishery, whose management effects are already affected by climate change (Kjesbu *et al.*, 2014). Using laboratory experiments we were able to show in Stiasny *et al.* (2016) that larvae of the North-East Arctic cod stock showed significantly increased mortality rates after hatch under end-of-the-century acidification. This can have significant effects on recruitment of this population (Stiasny *et al.*, 2016; Königstein *et al.*, 2017). In this study we aim to further explore whether the remaining individual larvae that had survived the treatment at 35 to 36 days post-hatching were nevertheless affected by acidification. To do so we compared the whole body and the organ response at two different prey densities and measured changes in size, dry weight, skeletal and organ development (liver, eyes, pancreas, kidney, gills) in relation to energy limitation were analyzed for the evaluation of potential trade-offs in growth and development responses caused by ocean acidification.

3.3 Methodology

The experiment was performed in 2014 at the then Norwegian National Cod Breeding Centre, now named Centre for Marine Aquaculture, in Tromsø, Norway. In order to obtain eggs and larvae, adult cod were caught alive in the Barents Sea at roughly 70°15’N, 19°00’E in March 2014 and were transferred to the Centre in Tromsø. They were kept in large breeding tanks (25 m^3) at ambient temperature, light and CO_2 conditions. Spawning occurred naturally in the breeding tanks and all eggs were collected from the outflow. These were transferred to incubators with either ambient (503 ± 89 µatm CO_2) or increased CO_2 (1179 ± 87 µatm) concentrations. Egg developmental stages were examined under a stereomicroscope to ensure that several females participated in these spawning events (Fridgeirsson, 1978). At 100% hatch occurring at 112 degree-days, 11,000 larvae were transferred into each of the twelve 190 L rearing tanks with a constant flow-through of water from two header tanks, six tanks each were supplied from a header tank with ambient water and from a header tank containing water with increased CO_2 concentrations. For the egg incubation and the start of the experiment the temperature was set to 6°C and was later, in the larval tanks, raised to 10°C in all tanks at constant light conditions (24h). Larvae were fed with *Nannochloropsis* and *Brachionus* until day 29 after which feeding was switched to Artemia nauplii. The high food treatment tanks were fed seven times a day and the low food treatment were fed three times a day. Prey concentrations fed at each feeding were the same for both treatments (Table 3.5).

Larvae in one tank in the ambient CO_2 treatment were abruptly lost over night, due to an unknown factor, resulting in six replicates for the high CO_2 treatment and five for the ambient treatment. This study was carried out in strict accordance with the laboratory regulations applicable in Norway. The application was approved by the National Regulatory Committee on the Ethics of Animal Experiments (Permit FOTS id 6382). All conditions and sampling were conducted to minimize suffering.

Ambient (pCO$_2$: 503 ± 89 µatm; pH 7.9 ± 0.15) and increased CO_2 (pCO$_2$: 1179 ± 87 µatm; pH 7.61 ± 0.03) levels were achieved by controlling the pH values in a header tank with pH probes connected to an IKS computer system. If the values deviated from the set target pH, a magnetic valve was opened, which allowed a pulse of CO_2 from a storage bottle to go into the inflow of the header tank. The volume of the header tank ensured a thorough mixing and equilibration of CO_2 before the water entered the rearing tank thereby assuring constant conditions in the rearing tanks. The pH and temperature was furthermore manually checked every day in the rearing tanks with a separate pH/temperature probe (WTW pH/Cond 340i/3320). Water chemistry, including DIC and alkalinity, was tested weekly based on the Best Practices Guide (Riebesell *et al.*, 2010). For further details please consult the Supplementary Information of Stiasny *et al.* (2016).

For the growth measurements larvae were sampled alive, anaesthetized with MS222 (Ethyl 3-aminobenzoate methanesulfonate) and frozen at -20°C. The sampled larvae were later photographed under a stereomicroscope next to a micrometer scaling bar. The photographs were then used to measure the larvae using the software ImageJ. In order to measure dry weight, larvae were freeze-dried (Christ Alpha 1-4 freeze dryer, Martin Christ Gefriertrocknungsanlagen GmbH, Osterrode, Germany) before being weighed (Sartorius SC2 microbalance, Sartorius AG, Göttingen, Germany, precision 0.1µg).

Table 3.5. Details & concentrations of feeding schedule of both food treatments.

Low Food						High Food				
DPH	Greenwater/ Nannochlorops	Rotatoria per Tank per Day(mill.)	Number of daily feedings of Rotatoria	Artemia per Tank per Day(mill.)	Artemia-number of daily feedings	Greenwater/ Nannochloropsis	Rotatoria per Tank per Day(mill.)	Number of daily feedings of Rotatoria	Artemia per Tank per Day(mill.)	Artemia-number of daily feedings
1	yes	4.27	7			yes	4.27	7		
2	yes	4.27	7			yes	4.27	7		
3	yes	4.27	7			yes	4.27	7		
4	yes	4.27	7			yes	4.27	7		
5	yes	7.35	7			yes	7.35	7		
6	yes	7.35	7			yes	7.35	7		
7	yes	7.35	7			yes	7.35	7		
8	yes	7.35	7			yes	7.35	7		
9	yes	7.35	7			yes	7.35	7		
10	yes	7.35	7			yes	7.35	7		
11	yes	7.35	7			yes	7.35	7		
12	yes	7.35	7			yes	7.35	7		
13		7.35	7				7.35	7		
14		3.15	3				7.35	7		
15		3.15	3				7.35	7		
16		3.15	3				7.35	7		
17		3.15	3				7.35	7		
18		3.15	3				7.35	7		
19		3.15	3				7.35	7		
20		3.15	3				7.35	7		
21		3.15	3				7.35	7		
22		3.15	3				7.35	7		
23		3.15	3				7.35	7		
24		3.15	3				7.35	7		
25		2.28	3	0.51	3		5.35	7	1.19	7
26		2.28	3	0.51	3		5.35	7	1.19	7
27		2.28	3	0.51	3		5.35	7	1.19	7
28		2.28	3	0.51	3		5.35	7	1.19	7
29		2.28	3	0.51	3		5.35	7	1.19	7
30				0.53	1				0.80	3
31				0.53	1				0.80	3
32				0.53	1				0.80	3
33				0.53	1				0.80	3
34				0.53	1				0.80	3

For the ossification analysis, all specimens were fixed in 70% ethanol (Schnell *et al.*, 2016). For the investigation of the skeletal development, the larvae were cleared and double stained (c&s) in an acid-free c&s method, following a modified protocol of Walker & Kimmel (2007). After fixation specimens were stained for cartilage in 6 ml of an acid-free alcian blue staining solution corresponding to Part A of Walker & Kimmel (2007). After 24 hours the specimens were washed in a 70% ethanol solution to remove excessive alcian blue and transferred for another 24 hours into 6 ml of a 0,5% KOH solution containing four droplets of a 3% H_2O_2 solution and alizarin red powder. The solution should have a dark purple coloration in order to obtain good staining results. During these 24 hours the tissues of the larvae were cleared by KOH, bleached by H_2O_2 and stained for calcified structures by alizarin red. Finally the specimens were transferred into a 70% glycerin solution for dissection and digital documentation. All specimens were digitally photographed with an Axiocam microscope camera attached to a ZEISS Discovery V20 stereomicroscope and processed with the Zeiss ZEN software. The number of ossified vertebrae was counted on these photographs. The branchial basket of each larva was removed and the third ceratobranchial was dissected out and photographed in order to measure the ossified structure (the pink stained structure in Figure 3.1) in length (horizontal line in Figure 3.1a), the length of the longest gill filament (vertical line in Figure 3.1a) and the area of gill filaments above the ossified part of the ceratobranchial (as shown in red in Figure 3.1b).

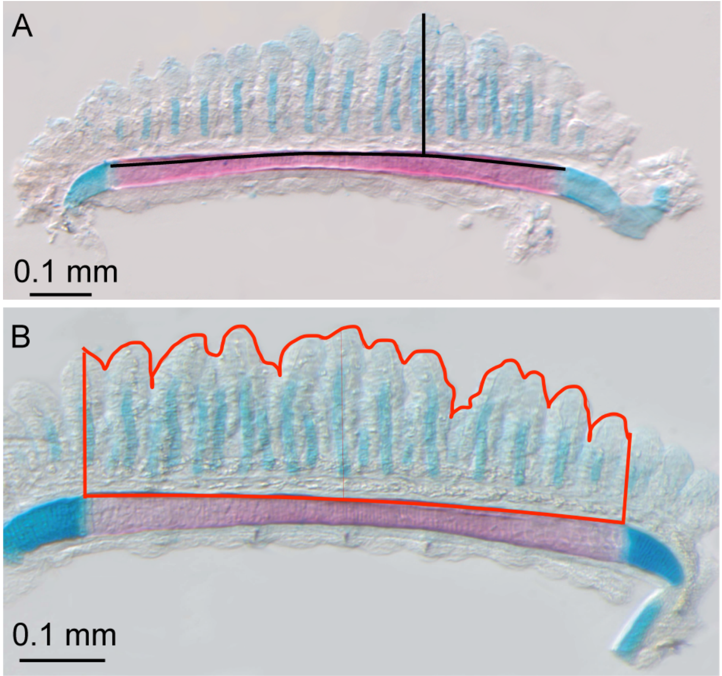


Figure 3.1 Third gill branchiale with gill filaments showing the (a) measured lengths of the ossified gill arch (horizontal line), which is stained in pink, and the longest gill filament (vertical line) and (b) the measured gill filament area (shown encircled in red).

Larvae for the histological analysis were fixed in 4% buffered formaldehyde at 35 dph, embedded in *Technovit*® or paraffin, sectioned transversely or longitudinally respectively at 3µm, followed by staining with methylene blue or haematoxylin and eosin respectively. *Technovit*-sections from head region (with eyes, gills and heart), front part of gut (with liver, pancreatic tissue, kidney tissue) as well as paraffin sections were studied and photographed with the microscope (Leitz Aristoplan with a Leica DFC295 camera). Moderate or numerous amounts of vacuoles in the pigment layer of the retina were noted and given a subjective score from + to +++ (some-several-many). A similar score was used for registrations of lipid vacuoles in the cod larvae livers.

RNA/DNA ratios reflect the relative condition and growth potential of fish larvae, due to the fact that while DNA content is stable in the cell, the RNA content, representing the protein biosynthesis machinery of the cell, varies with the nutritional state of the larvae (Clemmesen, 1994). Prior to RNA/DNA analyses and lipid determination all larvae previously stores at -80°C were freeze dried for 16 hours (Christ Alpha 1-4 freeze dryer, Martin Christ Gefriertrocknungsanlagen GmbH, Osterrode, Germany) and weighed (Sartorius SC2 microbalance, Sartorius AG, Göttingen, Germany; precision 0.1 µg). Measurements of RNA and DNA were made using the fluorometric method described by Clemmesen (1993) and modified by Malzahn *et al.* (2007). 5 larvae from 3 replicate tanks each were freeze dried, weighed and the tissue was homogenized in 400 µl or 800 µl 0.01% sodiumdodecyl sulfate Tris buffer (TE SDS), depending on the dry mass. When necessary, the homogenate was diluted up to 10-fold with 0.01% TE-SDS prior to fluorimetric determination. Ethidium bromide was used as a specific nucleic acid fluorescent dye for both RNA and DNA, and the total fluorescence was measured (Fluoroskan Ascent, Thermo Scientific, Waltham, Massachusetts,USA). RNAse was then used to digest all RNA enzymatically. The RNA fluorescence was calculated by subtracting the DNA fluorescence from the total fluorescence. By using the calibration curve fitted to the standard measurements (23 s r-RNA Boehringer, Boehringer Ingelheim GmbH, Ingelheim am Rhein, Germany) the amount of RNA was calculated. Following Le Pecq & Paoletti (1966), the DNA concentration was calculated using the relationship between RNA and DNA fluorescence with a slope ratio of standard DNA to standard RNA of 2.2, which adjusts for the relative fluorescence intensity difference of RNA and DNA.

Total lipids were extracted from individual freeze-dried and weighted cod larvae (5 additional larvae from each tank) using a modification of the Folch method (Folch *et al.*, 1957) with dichlormethan/methanol/chloroform (1:1:1 v/v/v). Freeze dried larvae were individually placed in 1.5 ml of the solution in a glass vial, securely capped with Teflon lined screwcaps and stored at -80°C for 72 hours. The defatted carcasses were transferred into Eppendorf vials and placed with open lids in a desiccator for 48 hours to allow for evaporation of the remaining lipid solvents before determining the defatted dry weight on a microscale (Sartorius SC2 microbalance). By subtracting the dry weight of the defatted carcass from the total larval dry weight, the amount of lipids could be gravimetrically determined and presented as lipids in % of dry weight or as µg lipids/mg dry weight.

All statistical analyses were run in the programs R (Version 3.3.2) (R Core Team (2016). R: A language and environmental for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.R-project.org) and RStudio (Version 1.0.136) (RStudio Team (2015). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA, USA, www.rstudio.com). Graphics were done in the R package ggplot2 (H.Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer Verlag New York, 2009).

For the analysis two-way ANOVAs were used to test for interactions between the CO_2 and the food treatment. Transformations were used to achieve normality of the residuals whenever necessary and appropriate. Since several larvae per tank were used for analyses, the tank effect was checked for before every analysis. However, the tank never had a significant effect on the parameters.

3.4 Results

Averaged over acidification treatments, larvae in the high food treatment were significantly longer (df=1, F=156.3, $p<0.001$) and heavier (df=1, F=277.6, $p<0.001$) than those in the low food treatment. Additionally there was a significant interaction with the CO_2 treatment (SL: df=1, F=27.4, $p<0.001$; DW: df:1, F=16.99, $p<0.001$). While larval size under high food availability was not affected by the acidification, larvae in the low food treatment were significantly larger under high CO_2 compared to control CO_2 levels (Figure 3.2).

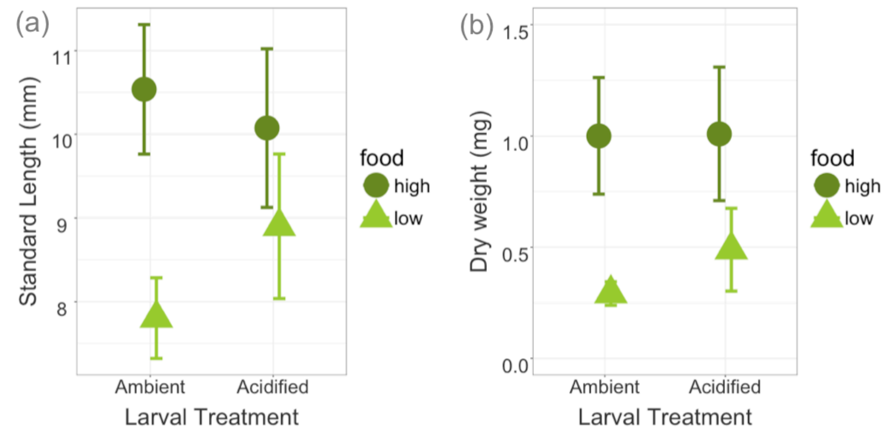


Figure 3.2. (a) Standard length in mm and (b) Dry weight in mg. Shown are mean values +/- standard deviation. The darker circles show the high food treatment and lighter triangles the low food treatment. (N=11-26 per treatment)

Under ambient CO_2 larvae in the high food treatment had nearly 400% more fully ossified vertebrae than those in the low food treatment. However, larvae in the low food, high CO_2 treatment had significantly more fully ossified vertebrae than those in the low food, ambient CO_2 treatment. In the high CO_2 treatment, the number of fully ossified vertebrae was highly similar between high and low food treatment larvae. (CO_2: df=1, F=13.1, p=0.001; food: df=1, F=21.2, $p<0.0001$; CO_2*food: df=1, F=26.3, $p<0.0001$) (Figure 3.3)

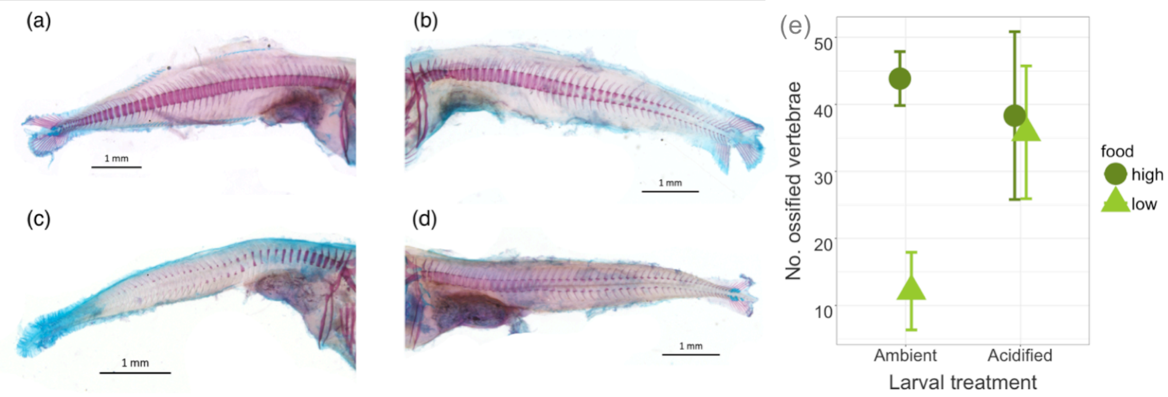


Figure 3.3. Schematic of number of ossified vertebrae (coloured in red) on 36 days-post-hatching in (a) ambient CO_2, high food (b) acidification, high food (c) ambient CO_2, low food (d) acidification, low food. Mean number of fully ossified vertebrae per treatment shown in (e). Shown are mean values +/- standard deviation. The darker circles show the high food treatment and lighter triangles the low food treatment. (N=7-12 per treatment)

The RNA/DNA ratio and the lipid content were also not significantly different between the CO_2 treatments in the high food treatment, but did differ between food treatments (df=1, F=244.4, p<0.0001) with overall lower RNA/DNA ratios in the low food treatment. The interaction between treatments was also significant (df=1, F=5.4, p=0.02). Larvae in the acidified treatment showed higher RNA/DNA than those in the ambient CO_2 treatment under low food conditions (Figure 3.4a). The lipid content (Figure 3.4b) was on average between 18 and 19% in all larvae from the high CO_2 treatment and in those from the ambient CO_2, high food treatment, but significantly higher in the larvae from the ambient CO_2, low food treatment (CO_2*food: df=1, F=13.6, p<0.001).

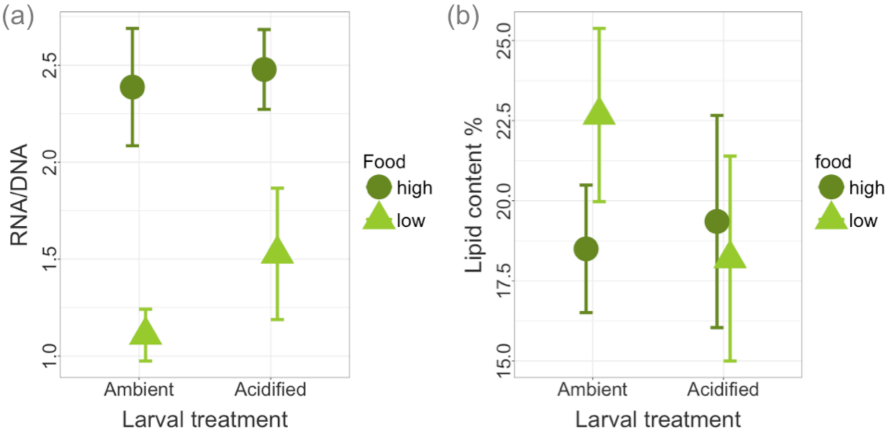


Figure 3.4. (a) RNA/DNA content (N=10-18 per treatment) and (b) Lipid content of the larvae (N=11-19 per treatment). Shown are mean values +/- standard deviation. The darker circles show the high food treatment and lighter triangles the low food treatment.

The length of the ossified gill arch was significantly affected by the food treatment (df=1, F=21.9, p<0.001) with longer ossified arches in the high food treatment (Figure 3.5a). The CO_2 treatment showed a significant interaction (df=1, F=8.1, p<0.01) with the food treatment. Larvae in the low food treatment have longer ossified arches in the high CO_2 treatment than those in the ambient treatment. The length of the longest gill filament was significantly affected by both treatments (CO_2: df=1, F=22.1, p<0.001; food: df=1, F=5.9, p=0.02) and there was no significant interaction. The gill filament was always longer in the ambient CO_2 treatment and in the high food treatment. (Figure 3.5b) The gill area was significantly affected by both treatments and their interaction (CO_2: df=1, F=11.3, p<0.01; food: df=1, F=14.9, p<0.001; CO_2*food: df=1, F=8.8, p<0.01) with larger gill areas shown by the larvae in the high food compared to the low food treatment in the larvae in the ambient CO_2 treatment. Larvae in the high CO_2 treatment were always similar to those in the ambient CO_2, low food treatment independent of their own food treatment. (Figure 3.5c)

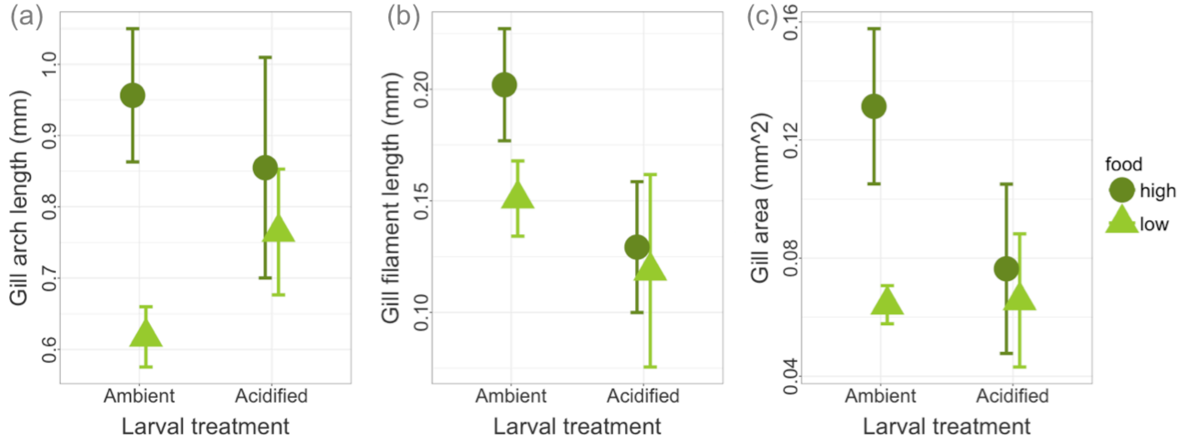


Figure 3.5. (a) Length of the ossified gill arch (mm) (b) length of the longest gill filament (mm) (c) gill area (mm²). Shown are mean values +/- standard deviation. The darker circles show the high food treatment and lighter triangles the low food treatment. (N=5-8 per treatment)

The histological samples show different severities of impairments in the organs. While vacuoles in the eyes were found across all treatments, vacuoles in the pancreas, the kidneys and particularly in the liver were found most commonly in the acidified, low food treatment. Impairments in the pancreas and the kidneys were mild, but larvae from the acidified, low food treatment showed partly severe to very severe vacuolization. (Figure 3.6)

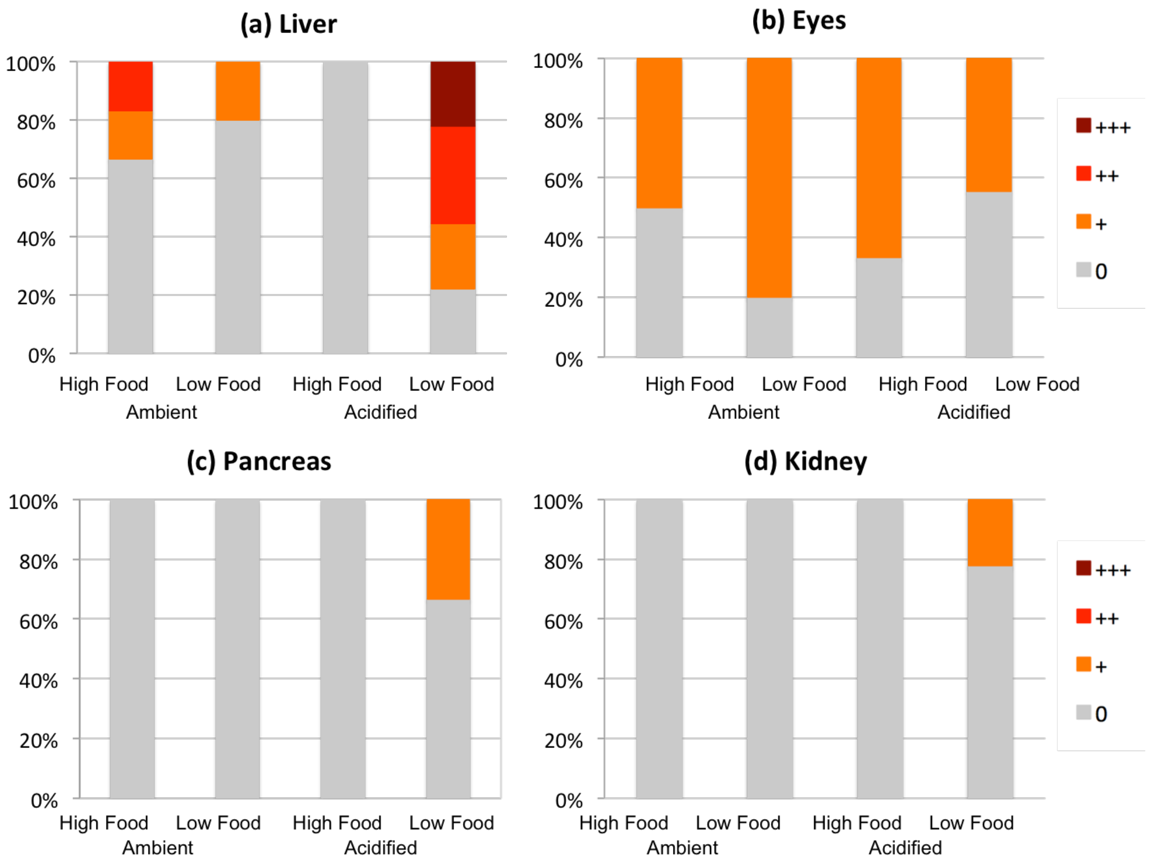


Figure 3.6. Frequency of organ impairments in (a) liver, (b) eyes, (c) pancreas, and (d) kidney of the cod larvae in a subjective scale from 0 (no impairments) to +++ (very severe/many impairments). (N=3-9 per treatment)

3.5 Discussion

Under realistic end-of-the-century CO_2 concentrations (Denman *et al.*, 2011; AMAP, 2013) a significant interaction between prey availability and acidification on the growth and development of the Arcto-Norwegian cod larvae became apparent. The high food treatment provided prey organisms *ad libitum*, which resulted in larger larvae in better higher nutritional condition, as evident in the higher RNA/DNA ratios. Nearly all vertebrae were ossified at 36 days post-hatching. In larvae in the high food treatment increased CO_2 concentration did not result in significant differences for these parameters, nor for lipid content in the larvae or the length of the ossified gill arch.

However, larvae in the low food treatment showed significant differences according to the CO_2 treatment. Larvae were heavier, longer, had higher RNA/DNA ratios, lower lipid contents and longer ossified gill arches in the acidified treatment compared to the ambient CO_2 treatment, when kept in low food in contrast to the expectation since these larvae should be more energy limited. In fact, larvae in the acidified treatment showed less difference between food treatments compared to the ambient CO_2 treatment. Larvae from the low food treatment exhibited similar growth and skeletal development than those raised under high food treatment when experiencing high CO_2 concentrations. The noticeable exception is found in organ development. Other organs, particularly the liver, were heavily impaired in the low food, acidified treatment, but not in the high food, acidified treatment. One possible explanation is the trade-off that the larvae in the low food treatment ultimately have to make since they are energy limited. It appears that they spend more energy on growth and ossification of skeletal elements in the acidified treatment compared to those larvae in the ambient treatment. The lower lipid content might be due to the process of ossification, since dietary lipids are important for skeletal development in marine fish (Lall & Lewis-McCrea, 2007; Kjørsvik *et al.*, 2009). Additionally these lipid levels might indicate that larvae in the low food, ambient treatment are still in an earlier developmental stage than the other treatments. This is also supported by the smaller larval size and fewer ossified vertebrae in this treatment. The organ impairments of liver and gills reflecting observations already shown by in cod, herring, tuna and summer flounders (Frommel *et al.*, 2012, 2014, 2016; Chambers *et al.*, 2013) may show the downside of the trade-off. The gill size was always smaller in the acidified treatment, independently from the food treatment, even though larval size was increased in the low food treatment. Considering that the active surface of the gill is a complex three dimensional structure and therefore several fold larger than the dimensional area that was measured (Lefevre *et al.*, 2017), these differences likely result in a very significant difference in functionality. The apparent decoupling of larval size to gill size may have a huge effect on the fitness of the larvae in the following weeks to months.

Increased calcification of the otoliths due to acidification has been shown for some species (Hurst *et al.*, 2012; Bignami *et al.*, 2013; Maneja *et al.*, 2013; Pimentel *et al.*, 2014; Réveillac *et al.*, 2015). This is likely due to the increased concentration of bicarbonate ions in the blood, which is elevated as a buffering mechanism during hypercapnia or pH stress (Melzner *et al.*, 2009a). The skeleton of marine fishes on the other hand is made of calcium phosphate, not calcium carbonate like the otoliths. The exact interaction remains unknown, but it appears that the ossification of skeletal elements is also affected by pH stress. Pimentel *et al.* (2014) found skeletogenesis to be defected in the larvae of *Solea senegalensis*. In contrast to the cod larvae in this study, a large proportion of larvae of *Solea senegalensis* showed severe deformities under increased CO_2 concentrations of a similar level.

While our study confirms larval size may increase under certain conditions under ocean acidification, it is premature to associate this finding with increased fitness of larvae. To the contrary, we found that the full picture of developmental processes is far more complicated, and larger larvae may carry more subtle bone and organ damaged. Increased larval size may through energetic trade-offs results in developmental patterns that can possibly decrease fitness of the larvae through decreased functionality of the organs or even impairments. Is so far remains unclear, what drives this trade-off and pushes the larvae to invest so heavily in growth.

To this day many studies have found negative effects of ocean acidification on fish larval size. Only few studies have looked at the effect of ocean acidification on whole body and organ level in relation to prey density. Baumann *et al.* (2012) found reduced standard lengths in the Atlantic silverside larvae (*Menidia beryllina*) with increasing acidification levels. A similar negative correlation between CO_2 concentration and larval size was found for the yellowfin tuna (*Thunnus albacares*) (Bromhead *et al.*, 2015) and Atlantic herring (*Clupea harengus*) (Frommel *et al.*, 2014). Other species, like juvenile scup (*Stenotomus chrysops*) (Perry *et al.*, 2015) or walleye pollock larvae (*Theragra chalcogramma*) (Hurst *et al.*, 2013) showed little to no effect of CO_2 on growth. Frommel *et al.* (2012) showed increased larval growth in Atlantic cod during some period of development, though at much higher CO_2 concentrations of above 4000 µatm. The orange clownfish *Amphiprion percula* similarly showed increased larval growth until settlement under increased CO_2 concentrations, although this effect differed between the larvae of different parents (Munday *et al.*, 2009b).

Although the number of studies on ocean acidification effects on marine fishes has steadily increased, the available data does not allow for a general conclusion on the responses relating to growth and development in marine temperate fish species due to very different experimental setups, life stages (often limited to very early life stages, like embryos, non-feeding larvae) analyzed and short term versus long term effects. Evaluations of benefits and trade-offs need a wide suite of different response parameters not always available.

3.6 Acknowledgements

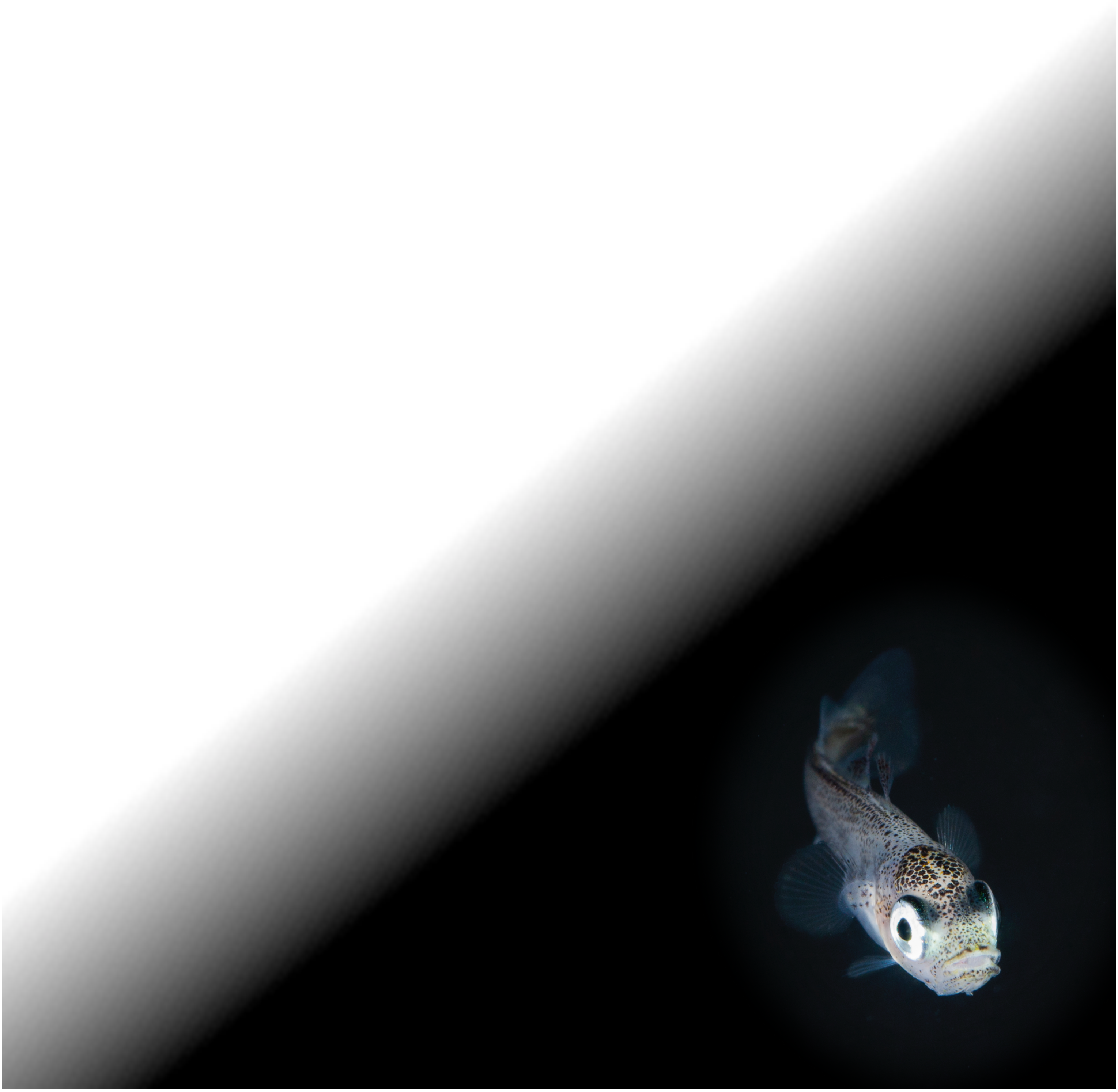
Funding was provided through the BIOAcid project (Biological Impacts of Ocean ACIDification) funded by the German Ministry for Education and Research (BMBF) (Grant No. 03F0655A), the AquaExcel transnational access grant for aquaculture infrastructures (Grant No. 0104/06/08/21) and the Bonus Baltic Sea research and development programme (Art 185) BIO-C3 project, funded jointly by the EU and the BMBF (Grant No. 03F0682A). The experiment was conducted at the Norwegian Cod Breeding Centre, NOFIMA, Tromsø, Norway. The authors are grateful to all scientists and staff at the station and all scientists from BIOAcid, who supported the experiment.

Additional information

The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to C.C.

4 MANUSCRIPT III

Parental Acclimation Effects in Response to Ocean Acidification in Atlantic Cod



Parental Acclimation Effects in Response to Ocean Acidification in Atlantic Cod

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Submitted to Scientific Reports

4.1 Abstract

Ocean acidification, the dissolution of excess anthropogenic carbon dioxide in ocean waters, poses a threat to many marine fish species (Dixson *et al.*, 2010; Munday *et al.*, 2010; Baumann *et al.*, 2012; Frommel *et al.*, 2012; Chambers *et al.*, 2013; Stiasny *et al.*, 2016). Whether species have potential to acclimate and adapt to changes in the seawater carbonate chemistry is still largely unanswered. Experiments across several generations are challenging for large commercially exploited fish species because of their long generation times. For Atlantic cod (*Gadus morhua*), we present first data on the effects of parental acclimation to enhanced CO_2 on larval survival in the F1 generation, a fundamental parameter determining population growth. The parental generation was exposed to either ambient or elevated CO_2 levels simulating end-of-century OA levels (~1100 µatm CO_2) for six weeks prior to spawning. Upon fully reciprocal exposure of the F1 generation, we quantified larval survival, combined with two feeding regimes in order to investigate the potential effect of energy limitation. We found a significant reduction in larval survival due to acidification that was, however, partly compensated by parental acclimation to ocean acidification. Such compensation was only observed in the treatment with high food availability to the larvae. This complex 3-way interaction indicates that surplus metabolic resources need to be available to realize a transgenerational alleviation response to ocean acidification.

4.2 Article

Atlantic cod (*Gadus morhua*) supports large, commercial fisheries in many areas around the Northern Atlantic. The different stocks already span a wide range of environments in terms of temperature and are predicted to react differently to changing temperatures, depending on where they already exist in relation to their thermal optimum. The most Northern stocks are believed to benefit from warming (Drinkwater, 2005) at least to a certain degree, through range expansion and through direct positive effects of slightly increasing temperatures on recruitment and growth. However, these populations will likely be most vulnerable to ocean acidification since changes in seawater pH are predicted to be greatest in the Arctic (Steinacher *et al.*, 2008). Hopes that global warming might have positive effects on the fisheries in these areas, as might have previously been the case (Kjesbu *et al.*, 2014), might therefore be ill placed, considering the accumulating evidence that ocean acidification negatively effects many species (Baumann *et al.*, 2012; Frommel *et al.*, 2012, 2014; Stiasny *et al.*, 2016). Progress has been made on research on the potential for acclimation and adaptation of other populations to acidification (Sunday *et al.*, 2014), but the role of non-genetic transfer of information via transgenerational effects is still under debate.

To this day, only few studies have addressed, whether and how much transgenerational acclimation might affect fitness-relevant traits or population vital rates of offspring. Most evidence comes from tropical reef fish, namely the Spiny Chromis (*Acanthochromis polyacanthus*) (Miller *et al.*, 2012; Welch *et al.*, 2014), the Fire Clownfish (*Amphiprion melanopus*) (Allan *et al.*, 2014), or the stickleback (*Gasterosteus aculeatus*) (Schade *et al.*, 2014; Shama & Wegner, 2014; Shama *et al.*, 2016). The results on the effect of transgenerational acclimation to ocean acidification from these studies cannot be generalized at the moment. Exploring the potential of fish populations to adapt to ocean acidification through multi-generational experiments, as it has been done with coral reef fishes (Donelson *et al.*, 2011), is unfeasible for most temperate and commercial species, because of their long generation times, larger body size and low survival of eggs and larvae in each generation (e.g. Atlantic cod 3-5 years). Rummer & Munday (2016) conclude that there is evidence for potential acclimation and adaptation in coral reef fishes, because even though experimental data suggests a negative effect of temperature and acidification, reef fish populations already exist at temperatures above those predicted by climate change. Similarly hopeful information is not available for most commercial, temperate species. On the contrary, we have shown in Stiasny *et al.* (2016) that Atlantic cod populations from the Western Baltic and the Barents Sea reacted surprisingly similar to ocean acidification. Daily mortality rates doubled in both populations, even though the Baltic stock has a history of already experiencing low pH due to upwelling events in the Western Baltic (Melzner *et al.*, 2012). The hypothesis that Baltic species might be able to cope since they had time to adapt was therefore falsified.

In this study we exposed adult cod to either ambient seawater or seawater with increased CO_2 concentrations as they might be expected globally around the year 2100 following the IPCC RCP 8.5 (IPCC, 2013) (~1100 μatm) for six weeks prior to spawning. This coincides with the last stages of gonadal development and egg maturation. Resulting eggs and larvae were reared either in the parental CO_2 concentrations or the opposite treatment. Larval survival and growth were measured and histological samples of certain organs, including the eyes and the liver, were taken and analyzed. The tested hypothesis was that larvae, which came from parents, who already experienced ocean acidification during gonadal development, might cope better with these conditions due to possible acclimation of the parents and transgenerational effects.

Ocean acidification treatments were combined with two different feeding treatments – ad libitum vs. limited food availability - in a full factorial design with the acidification treatments. In order to test for the effects of energy limitations a low food treatment was chosen with the same prey densities in the tanks, in order to avoid effects due to different larval densities, but with significantly less feedings per day to investigate whether coping with CO_2 stress was energy dependent (for more information on the feeding regimes, please consult the methodology in SI).

In a completely independent data set, we were able to confirm enhanced larval mortality by experimental ocean acidification (Stiasny *et al.*, 2016). However, as hypothesized, the parental exposure to ocean acidification modified the immediate reaction of larvae. The transgenerational effect was buffering with high food availability, i.e. offspring of parents exposed to ocean acidification survived better under high food, and vice versa under low food, indicated by a significant three-way interaction (Figure 4.1). Larvae of parents acclimated to high CO_2 under high food/ high CO_2 showed survival rates intermediate between larvae in ambient seawater and those without prior exposure in the parental generation to increased acidification. This shows some compensatory mechanism of parental acclimation since survival improved compared to the direct, sudden exposure to CO_2 of larvae. However, the effect was not large enough to completely counteract the effect of acidification back to survival rates in the ambient treatment. Furthermore this compensatory effect was completely absent in the low food treatment. Here larvae exposed to high CO_2 coming from CO_2 acclimated parents showed even lower survival on day 16 post-hatching than those from non-acclimated parents. (Figure 4.1).

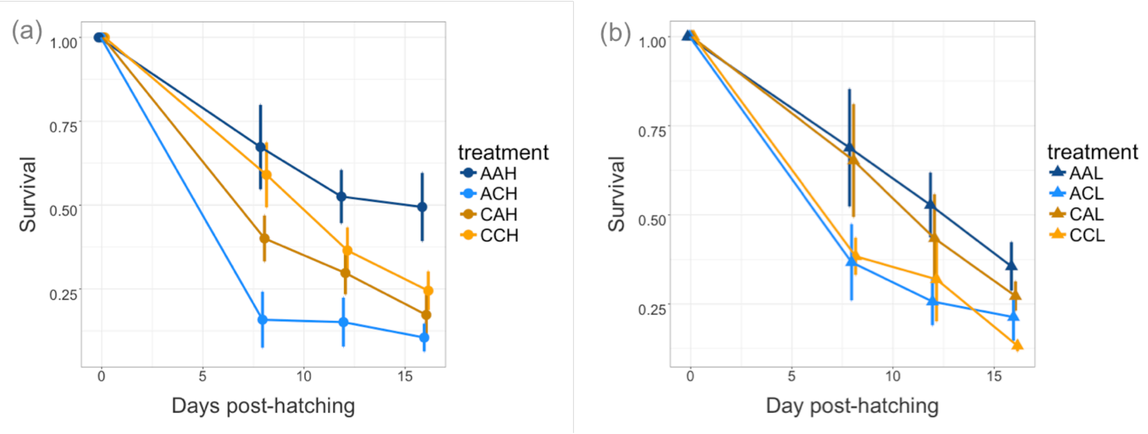

Figure 4.1. Survival of cod larvae from hatching to 16 days post-hatching in the high food treatment (a) and the low food treatment (b) depending on parental (1st letter, Ambient (A), high CO_2 (C)), larval (2nd letter) treatment and food (3rd letter, High (H), Low (L)) Shown are mean values and standard error across three replicates per treatment.

Considering that the low food treatment is likely still above natural prey concentrations in the field and match-mismatch situations of larvae hatching and prey concentrations might also increase due to climate change, this does not bode well for the future of cod larvae.

Larval growth in terms of dry weight and standard length at day 36 post-hatching was not affected by the CO_2 treatment of parents nor the offspring, but larvae in the low food treatment grew less ($F=43.795$, $p<0.0001$), indicating that they were indeed energy limited (Figure 4.2). Since experimental animals came from an aquaculture stock, bred for optimal growth, it is unlikely that this absence of an effect on the vulnerability to acidification is easily transferable to wild populations.

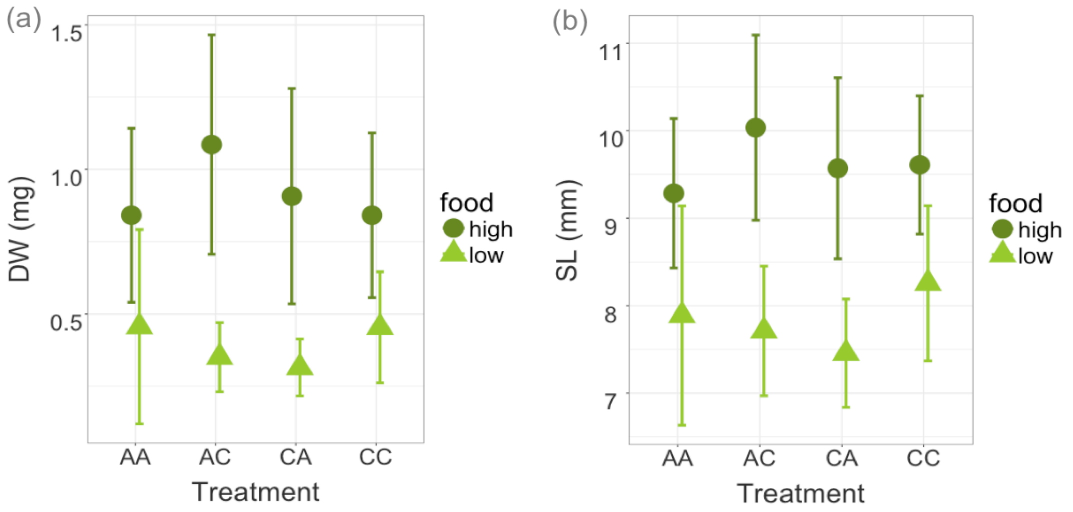

Figure 4.2. (a) Dry weight (in mg) and (b) Standard length (in mm) on 36 days post-hatching depending on parental (1st letter Ambient (A), high CO_2 (C)) and larval treatment (2nd letter) and food availability (high (dark circles) and low (lighter triangles). Shown are mean values and standard deviation of ten larvae times three replicates.

Under realistic end-of century ocean acidification levels, we found widespread histological damage suggesting impairments of major organ functioning. Particularly the larvae in the high CO_2 treatment, which came from acclimated parents, showed strong impairments more frequently independently of the food treatment (Figure 4.3 and 4.4). Vacuoles in the pigment layer of the retina of the 35 days old larvae were registered in all treatments, but were apparently more frequent in larvae from tanks with elevated CO_2 concentrations (Figure 4.3). Gill structure looked similar in all investigated larvae. Cartilage made up the supporting skeleton in both gill arches and filaments. Apparent similar heart morphology was also notes in all larvae. Pancreatic tissue was difficult to evaluate, but no common abnormalities were registered. A few larvae from both treatments had vacuoles in the tissue. Kidney tissue showed apparently normal tubuli and glomeruli in all groups. Liver morphology varied between individual samples and CO_2 regimes (Figure 4.4 D-L). Glycogen granules were noted in all livers sectioned (H), while numerous empty vacuoles (representing lipid inclusions) of variable sizes were characteristic of some of the CO_2 treated larvae (D-F, L). This phenomenon was also registered in some of the control larvae. But generally larvae from the ambient treatment had smaller and regular vacuoles

(G, H, I, K). Abnormal vacuolation in the liver may represent a functional problem (Frommel *et al.*, 2012, 2014). Hepatocyte vacuolation was not more frequently registered in the larval group in the high food compared to the low food treatment.

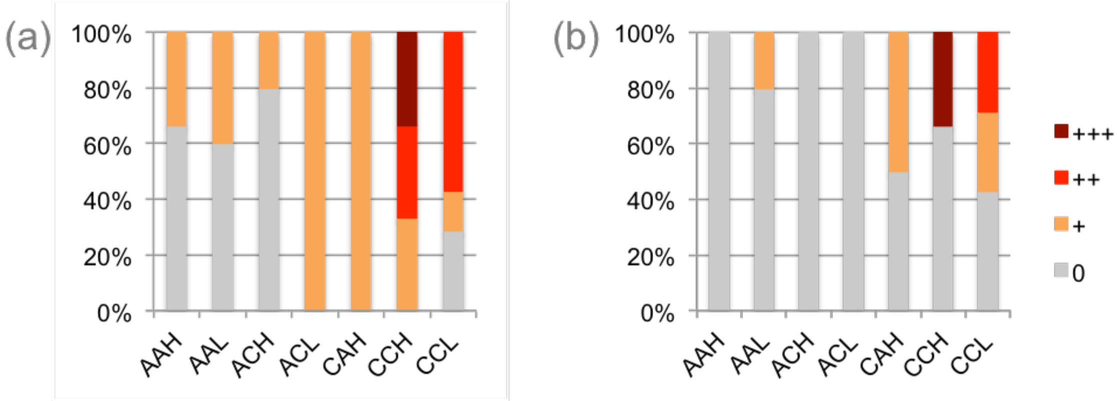

Figure 4.3. ***Frequency of (a) liver and (b) eye lipid vacuolization in subjective scores from 0 to +++ depending on parental treatment (1^{st} letter (A-Ambient, C-high CO_2)), larval CO_2 treatment (2^{nd} letter (A-Ambient, C-high CO_2)) and food treatment (3^{rd} letter, H – high food, L – low food). (N=1-7)***

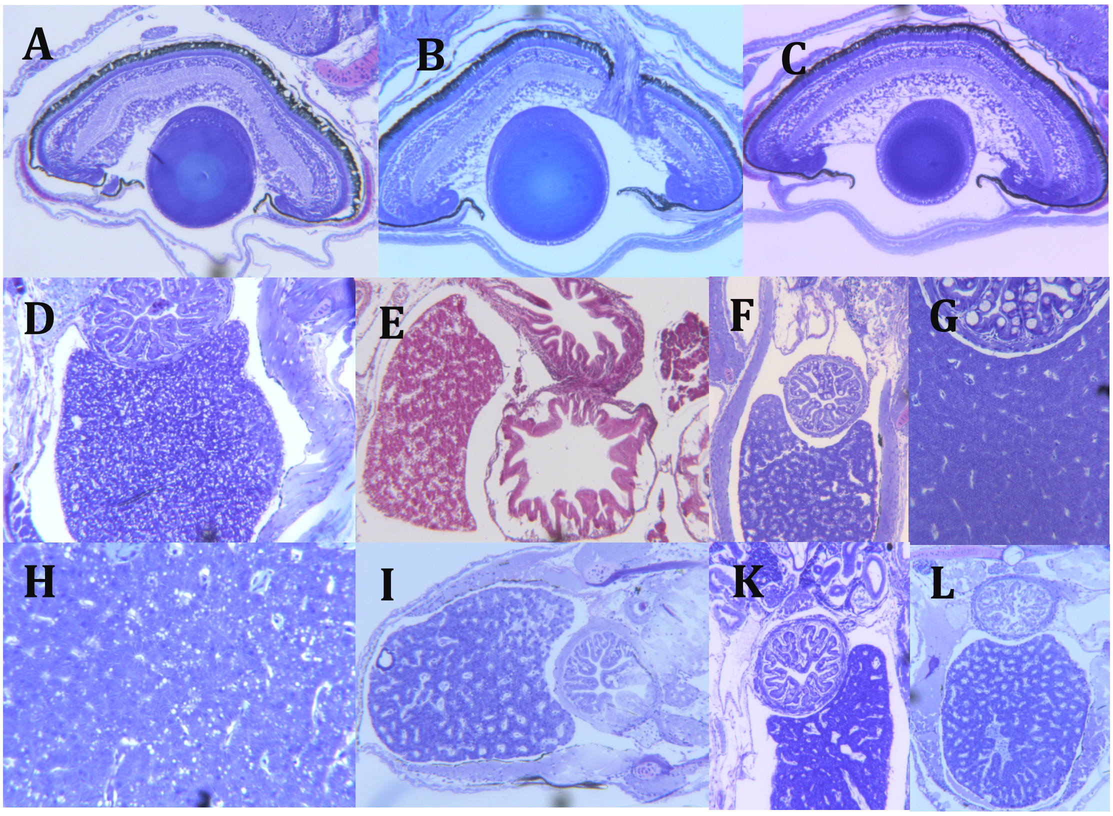

Figure 4.4. Examples of histological eye (A-C) and liver (D-L) samples from various treatments. A: Eye,CCH; B: Eye AAL; C: Eye AAH; D: Liver, CCL; E: Liver, CCH; F: Liver CCL; G: Liver, AAH; H: Liver, AAL; I Liver, AAL; K: Liver, AAH ; L: Liver, CCL

The histological results show clearly that in the high food treatment, where larval survival was partly compensated by the parental acclimation, larvae still suffered developmental impairments and organ damage under ocean acidification. The histological impairments due to acidification in this experiment were less pronounced than those found by Frommel *et al.* (2012, 2014). This is likely due to the more realistic, lower levels of carbon dioxide used in this experiment.

In this study on parental acclimation and transgenerational effects to ocean acidification on a commercial fish species, results show that under optimal food conditions, parental acclimation can have a compensatory effect on survival, however organ damages are not compensated, possibly even amplified. Additionally the compensation on survival is not strong enough to completely counteract the effect of ocean acidification and larval survival is still significantly lower than under ambient conditions. One reason for the incomplete compensation may be the limited timespan of parental acclimation of only 6 weeks. It is possible that a longer acclimation period or effects across several generations might produce a stronger transgenerational effect. Nonetheless the compensation appears to depend on the availability of prey and therefore energy. It is therefore unclear whether this is a mechanism that is likely to improve the situation for cod larvae in the wild. This study clearly demonstrates the complexity of acidification effects on physiology and on the potential for acclimation. The three included parameters of parental and larval treatments as well as food treatment show strong interactions, proving that predictions of exact effects will be extremely difficult to give. Nonetheless, even the most beneficial combination of the treatments results in strong impairments on the larval phase if these are exposed to ocean acidification.

4.3 Supplementary Information

4.3.1 Methodology

Parental treatment

Adult cod from the aquaculture stock of the company Nofima AS at the Centre for Marine Aquaculture (*Senter for marin akvakultur*, formerly the Norwegian Cod Breeding Centre *Nasjonal avlsstasjon for torsk*) in Tromsø, Norway were transferred from the sea cages to the Centre on 16th January 2014 to start the incubation. These aquaculture stocks were a mixture of two wild stocks, the Norwegian coastal cod and the North-East-Arctic (NEA) cod from the Barents Sea. These cod were third generation aquaculture organisms. They were kept in net cages in the fjord and were transferred using a well boat and transfer tanks. They were split into the two treatments in large storage tanks with a diameter of 4 m, filled with 18 m^3 seawater. They were kept in a constant seawater flow through of 225 l/m. The light regime was matched weekly to outside conditions.

Deep-water from the Tromsø Fjord was pumped directly from in front of the station and then filtered. Carbon dioxide concentrations in the acidified treatment were controlled by an IKS Aquastar System. CO_2 influx from a bottle is controlled via magnetic valves. The pH was additionally checked daily with a WTW pH 3310 hand probe with a *SenTix*® *H* pH-electrode. Water samples for carbonate chemistry were taken and analyzed at the University of Tromsø (See Stiasny et al., 2016 for more details on the carbonate chemistry in the experiment).

The adult cod were regularly checked for running eggs and sperm. When mature and running, they were strip-spawned and fertilized eggs were transferred into incubators. Larvae in each experimental treatment consisted of at least five to seven different families.

Egg and larval treatment

Fertilized eggs were transferred to incubators, which were kept at 6°C and were constantly aerated. After hatching the larval density was counted in the incubators and 11 000 larvae were transferred into each tank and the larval experiment was started. This day was set as 0 days post-hatching (dph), even though larvae had hatched over several days before.

The larval tanks were started at 6°C, but were later raised to 10°C in all tanks. Light was kept on constant at 24 hours a day. Larvae were fed with *Nannochloropsis* and *Brachionus* at different intervals for the different food treatments (seven in the high compared to three times daily in the low food treatment at the beginning and five times compared to one time daily later on). The prey concentrations given per feeding remained constant and the same for both treatments.

An IKS computer system and a pH sensor in the header tank, from which the water would flow to the rearing tanks after mixing, controlled the CO_2 concentrations. The pH was checked manually in the rearing tanks every day using a pH sensor (WTW pH/Cond 340i/3320) Water chemistry, including DIC and alkalinity, was tested weekly based on the Best Practices Guide (Riebesell *et al.*, 2010).

For more information on feeding concentrations and carbonate chemistry, please consult the article and the SI of (Stiasny *et al.*, 2016).

Survival measurements

Survival was measured three times in the larval tanks, starting on day 8 post-hatching, by measuring the density of remaining larvae. Five subsamples of 0.8 l were taken across the whole water column using a pipe, which could be closed at the bottom, and the number of living larvae in the subsamples was counted. An increased aeration during the sampling process ensured an even distribution of larvae in the rearing tanks. The accuracy of the method was repeatedly checked in separate tanks. After day 16 post-hatching of the experiment, the method became inaccurate and imprecise. This is likely due to the increased swimming ability of the larvae, combined with improving sensory abilities, which probably resulted in an uneven distribution of larvae in the tanks due to avoidance behaviour towards the pipe and the increased aeration. Survival data was therefore not usable after 16 dph, but larvae were sampled for growth and histology measurements until 36 and 35 dph.

Growth

Ten larvae per tank were sampled alive from each rearing tank. Larvae were euthanized using Tricaine methanesulfonate (Ethyl 3-aminobenzoate methanesulfonate, MS222) before being frozen. Later larvae were thawed and photographed next to a scale through a microscope. The photographs with the scales were used to measure the Standard Length of the larvae by measuring the length from the tip of the vertebrae, following the vertebrae and then an imaginary line from the front of the vertebrae through the eyes to the tip of the lower jaw. In order to measure dry weight, larvae were freeze dried before being weighed.

Statistical analysis

All statistical analyses were run in the program R (Version 3.3.2) and RStudio (Version 1.0.136). For growth measurements ten larvae per tank were sampled in order to get an accurate assessment of the variance and a larger sample size than N=3. To include the possibility of tank effects a linear mixed effects model was run to test for differences and interactions between the treatments, but also including tank as a random factor. Transformations were performed if necessary to achieve homogeneity of variance and normality of residuals.

Table 4.1. Results of the Statistical Analyses.

Tested parameter	**Factor**	**F value**	**p value**
Standard Length	Parental CO_2 treatment	0.475	0.4996
	Larval CO_2 treatment	1.115	0.3049
	Food treatment	40.940	<. 0001
Dry Weight	Parental CO_2 treatment	0.145	0.708
	Larval CO_2 treatment	0.424	0.523
	Food treatment	43.795	<.0001
Survival Day 16	Parental CO_2 treatment	2.816	0.114
	Larval CO_2 treatment	11.48	0.004
	Food treatment	0.001	0.976
	Parental*Larval Treatment	7.197	0.017
	Parental*Food Treatment	0.003	0.955
	Larval*Food Treatment	0.003	0.960
	Parental*Larval*Food Treatment	7.837	0.013

Histology

Larvae were fixed in 4% buffered formaldehyde at 35 dph, embedded in *Technovit*® or paraffin, sectioned transversely or longitudinally respectively at 3µm, followed by staining with methylene blue or haematoxylin and eosin respectively. *Technovit*-sections from head region (with eyes, gills and heart), front part of gut (with liver, pancreatic tissue, kidney tissue) as well as paraffin sections were studied and photographed in the microscope (Leitz Aristoplan with a Leica DFC295 camera). Moderate or numerous amounts of vacuoles in the pigment layer of the retina were noted and given a subjective score from + to +++ (some-several-many). A similar score was used for registrations of lipid vacuoles in the cod larvae livers.

MANUSCRIPT III

5 SYNTHESIS & PERSPECTIVE

5.1 Physiological effects of ocean acidification on Atlantic cod larvae

The results of my dissertation quantify for the first time the impact of ocean acidification on Atlantic cod larval survival and resulting changes in recruitment. The data reveal that acidification effects may be strong enough to significantly alter population dynamics and therefore affect entire stocks and fisheries. The results were remarkably similar for two cod stocks, the Baltic Sea stock and the Barents Sea stock.

Moreover, we were able to show that carbon dioxide concentrations predicted in the study areas within this century will have significant effects on larval physiology in terms of growth, as well as skeletal and organ development. The effects of food limitation on the capacity of the larvae to cope with the stress of hypercapnia had never been explored so far. It furthermore demonstrates that even when larvae are able to regulate their own pH and buffer acidification to a certain extent, this comes with a cost, most likely via an allocation trade-off, which seems to manifest for example in organ development.

The results on parental acclimation are the first to explore effects of ocean acidification across generations for a temperate, commercial species. We were able to demonstrate that parental acclimation has a significant effect on larval survival and organ development. However, it did not result in a complete offset of the impairments due to acidification in the larval generation, even under no food limitation and aggravated results when larvae were energy limited.

5.2 Integration into modelling efforts to explore changes in population dynamics, management strategies and economic impacts

The survival data in this dissertation are the first that offer the possibility to integrate ecophysiological responses to ocean acidification with population dynamics. The recruitment data from Manuscript I form the basis for several studies and papers, which are in the process of being published. Königstein, Dahlke, Stiasny, Storch, Clemmesen and Pörtner (2017) have developed an integrative model for the effects of ocean warming and acidification on the early life stages of Atlantic cod in the Barents Sea, termed SCREI – Simulator of Cod Recruitment under Environmental Influences. The survival data under direct ocean acidification exposure during the larval stage from this thesis are combined with data on egg fertilization and survival during the egg stage under increased temperatures and acidification from other partners within the project BIOAcid. Time series data on egg production, temperature, food and predator abundance are used for the calibration in order to project the recruitment success under different scenarios. The SCREI model enables estimations of uncertainties based on variation within the ecosystem and also between individuals and even although stochasticity is high in the population, the model predicts severe reductions in recruitment towards the middle to end of the century in the Barents Sea. Potential for adaptation would need to be quite high to counteract the impacts on the recruitment.

A population model on the Arcto-Norwegian Arctic cod, under development by Stiasny and Winter, integrates the direct effect of acidification and warming on the recruitment as well as temperature effects on the growth of the adult population in order to test the robustness of the management plan in terms of the harvest control rules, which are currently used for this stock. The negative effect of acidification on recruitment is initially buffered by a positive effect of temperature on recruitment, since the stock currently exists below the temperature optimum. However, the temperature optimum will be reached and likely crossed within this century. After this point temperature and acidification will both negatively affect the recruitment. Precautionary fishing levels are therefore highly likely to be lower than they currently are.

A study lead by Voss, Quaas, Stiasny et al. (submitted to the Journal of Applied Ecology) explores the effect of ocean acidification and temperature on the recruitment and economic viability of the Western Baltic cod stock. Temperature effects are taken from time-series data for this region. An ecological-economic optimization model was used to investigate the effect of acidification and warming on ecological (stock size), economic (profits), consumer-related (harvest) and social (fishing effort) indicators, ranging from present day conditions to future climate scenarios. Temperature and acidification both have a negative effect on the fishery in the Western Baltic and if both effects are combined, a viable fishery becomes impossible at a more than 1.5°C increase in temperature, even under optimal management. A main conclusion of this study is that current fisheries management and stock conditions are not sustainable and that fishing must be reduced in order to increase the spawning stock biomass. However, meeting of global climate targets is also vital.

5.3 Perspectives for future research

5.3.1 Open questions on ecophysiology responses

This dissertation has revealed further knowledge gaps in our understanding of ocean acidification on fish larval physiology. We were able to demonstrate that skeletal ossification is affected by the decrease in pH. This had previously been shown for the otoliths of fish larvae (Checkley *et al.*, 2009; Maneja *et al.*, 2013; Pimentel *et al.*, 2014). The hyper-calcification in otoliths is likely due to the increase in bicarbonate ions in the blood due to the buffering efforts of the organisms to keep the pH constant (Melzner *et al.*, 2009a). The effect on the ossification of the skeleton is not as directly linked to pH buffering, since it consists of calcium phosphate, rather than calcium carbonate. The current literature cannot resolve the link of hypercapnia to skeletal ossification.

Similarly it is unclear what the underlying physiological effects of acidification on organ development are. Manuscript II and III show impairments of particular organs via increased lipid vacuolization, particularly in the eyes and the liver. How hypercapnia is linked to the lipid metabolism remains unknown. In future studies it would be important to measure hormones, like cortisol, in the living larvae during the experiments in order to investigate possible mechanisms of the organisms to deal with the pH stress, which may explain differences in the metabolic processes.

The potential for acclimation and adaptation to ocean acidification is a rapidly expanding area of research and currently not much is known for long-lived fishes such as Atlantic cod. Manuscript III offers a first look into the effect of parental acclimation. The results show that larvae are affected by the exposure of the parental generation to acidification, however this also depended on their own food availability. Ideally Atlantic cod would need to be raised under high CO_2 concentrations for several generations in order to explore the long-term effect as well as transgenerational effects. This is practically very hard to manifest for such a large species with long generation times. Furthermore, it may be possible that husbandry effects could interact with the effects of acidification.

A separate PhD dissertation by Felix Mittermayer is currently looking into the transcriptome of the cod larvae during these experiments. So far the underlying mechanisms of how parental acclimation may affect the offspring remain unknown. This will provide some insights, which cellular and molecular processes are behind the physiological and histological effects that are shown in this dissertation.

5.4 Conclusion

The world's ocean is undergoing a series of fundamental changes and is facing a variety of threats, including increasing temperatures, pollution, deoxygenation, overfishing, and ocean acidification. At the same time the oceans are intrinsically linked to food security, human health and all other Sustainable Development Goals that the United Nations have set (United Nations General Assembly, 2015). Fish populations are one of the key links between the ecosystems, anthropogenic pressures and human welfare. They may be part of the answers to questions of sustainability, food security, development in third world countries, human health, and many more (International Council for Science, 2017). At the same time evidence is accumulating that they are themselves threatened by anthropogenic stressors, creating a vicious circle in the future world, which we need to break as soon as possible before it spirals out of control.

This dissertation has advanced our understanding of ocean acidification effects on larval physiology and population recruitment in Atlantic cod (*Gadus morhua*). The results have implications for the sustainable management of commercial fish stocks. While sustainability of fishing practices has always been imperative, the precautionary principle is gaining significance in the light of climate change, since additional pressures such as ocean acidification are now acting on the stocks.

6 BIBLIOGRAPHY

Allan BJM, Miller GM, McCormick MI, Domenici P, Munday PL (2014) Parental effects improve escape performance of juvenile reef fish in a high-CO_2 world. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 20132179.

AMAP (2013) *AMAP Assessment 2013: Arctic Ocean Acidification*. Oslo, Norway, viii+99pp..

Bailey KM, Houde ED (1989) Predation on Eggs and Larvae of Marine Fishes and the Recruitment Problem. *Advances in Marine Biology*, **25**.

Baumann H, Talmage SC, Gobler CJ (2012) Reduced early life growth and survival in a fish in direct response to increased carbon dioxide. *Nature Climate Change*, **2**, 38–41.

Bignami S, Enochs IC, Manzello DP, Sponaugle S, Cowen RK (2013) Ocean acidification alters the otoliths of a pantropical fish species with implications for sensory function. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 7366–70.

Bromhead D, Scholey V, Nicol S, Margulies D, Wexler J, Stein M, Hoyle S, Lennert-Cody C, Williamson J, Havenhand J, Ityina T, Lehodey P (2015) The potential impact of ocean acidification upon eggs and larvae of yellowfin tuna (*Thunnus albacares*). *Deep Sea Research Part II: Topical Studies in Oceanography*, **113**, 268–279.

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

Caldeira K, Wickett ME (2005) Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *Journal of Geophysical Research*, **110**, C09S04.

Chambers RC, Candelmo AC, Habeck EA, Poach ME, Wieczorek D, Cooper KR, Greenfield CE, Phelan BA (2013) Ocean acidification effects in the early life-stages of summer flounder, *Paralichthys dentatus*. *Biogeosciences Discussions*, **10**, 13897–13929.

Checkley DM, Dickson AG, Takahashi M, Radich JA, Eisenkolb N, Asch R (2009) Elevated CO_2 enhances otolith growth in young fish. *Science*, **324**, 1683.

Cheung WWL, Pinnegar J, Merino G, Jones MC, Barange M (2012) Review of climate change impacts on marine fisheries in the UK and Ireland. *Aquatic Conservation:*

Marine and Freshwater Ecosystems, **22**, 368–388.

Clemmesen C (1993) Improvements in the fluorimetric determination of the RNA and DNA content of individual marine fish larvae. *Marine Ecology Progress Series*, **100**, 177–183.

Clemmesen CM (1994) The effect of food availability, age or size on the RNA/DNA ratio of individually measured herring larvae : Laboratory calibration. *Marine Biology*, **118**, 377–382.

Cook RM, Sinclair A, Stefánsson G (1997) Potential collapse of North Sea cod stocks. *Nature*, **385**, 521–522.

Denman K, Christian JR, Steiner N, Pörtner H-O, Nojiri Y (2011) Potential impacts of future ocean acidification on marine ecosystems and fisheries : current knowledge and recommendations for future research. *ICES Journal of Marine Science*, **68**, 1019–1029.

Dickson AG (1990) Standard potential of the (AgCl(s) + 1/2H2(g) = Ag(s) + HCl(aq)) cell and the dissociation constant of bisulfate ion in synthetic sea water from 273.15 to 318.15 K. *The Journal of Chemical Thermodynamics*, **22**, 113–127.

Dickson AG, Millero FJ (1987) A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep Sea Research Part A. Oceanographic Research Papers*, **34**, 1733–1743.

Dickson AG, Sabine CL, Christian JR (2007) Guide to best practices for ocean CO_2 measurements. *PICES Special Publication*, **3**.

Dixson DL, Munday PL, Jones GP (2010) Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. *Ecology Letters*, **13**, 68–75.

Dixson DL, Pratchett MS, Munday PL (2012) Reef fishes innately distinguish predators based on olfactory cues associated with recent prey items rather than individual species. *Animal Behaviour*, **84**, 45–51.

Donelson JM, Munday PL, McCormick MI, Pitcher CR (2011) Rapid transgenerational acclimation of a tropical reef fish to climate change. *Nature Climate Change*, **2**, 30–32.

Doney SC, Fabry VJ, Feely RA, Kleypas JA (2009) Ocean Acidification: The Other CO_2 Problem. *Annual Review of Marine Science*, **1**, 169–192.

Drinkwater KF (2005) The response of Atlantic cod (*Gadus morhua*) to future climate change. *ICES Journal of Marine Science*, **62**, 1327–1337.

Falk-Petersen I-B (2005) Comparative organ differentiation during early life stages of marine fish. *Fish and Shellfish Immunology*, **19**, 397–412.

FAO (2016) *The State of World Fisheries in 2015. Contributing to food security and nutrition for all.* Rome, Italy.

Feely RA, Sabine CL, Lee K, Berelson W, Kleypas J, Fabry VJ, Millero FJ (2004) Impact of Anthropogenic CO_2 on the $CaCO_3$ System in the Oceans. **305**, 362–366.

Folch J, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, **226**, 497–509.

Franke A, Clemmesen C (2011) Effect of ocean acidification on early life stages of Atlantic herring (*Clupea harengus* L.). *Biogeosciences*, **8**, 3697–3707.

Fridgeirsson E (1978) Embryonic development of five species of gadoid fishes in Icelandic waters. *Rit Fiskideildar*, **5**, 1–71.

Frommel AY, Maneja R, Lowe D, Malzahn AM, Geffen AJ, Folkvord A, Piatkowski U, Reusch TBH, Clemmesen C (2012) Severe tissue damage in Atlantic cod larvae under increasing ocean acidification. *Nature Climate Change*, **2**, 42–46.

Frommel AY, Schubert A, Piatkowski U, Clemmesen C (2013) Egg and early larval stages of Baltic cod, *Gadus morhua*, are robust to high levels of ocean acidification. *Marine Biology*, **160**, 1825–1834.

Frommel AY, Maneja R, Lowe D, Pascoe CK, Geffen AJ, Folkvord A, Piatkowski U, Clemmesen C (2014) Organ damage in Atlantic herring larvae as a result of ocean acidification. *Ecological Applications*, **24**, 1131–1143.

Frommel AY, Margulies D, Wexler JB, Stein MS, Scholey VP, Williamson JE, Bromhead D, Nicol S, Havenhand J (2016) Ocean acidification has lethal and sub-lethal effects on larval development of yellowfin tuna, *Thunnus albacares*. *Journal of Experimental Marine Biology and Ecology*, **482**, 18–24.

Haigh R, Ianson D, Holt CA, Neate HE, Edwards AM (2015) Effects of Ocean Acidification on Temperate Coastal Marine Ecosystems and Fisheries in the Northeast Pacific. *Plos One*, **10**, e0117533.

Heath MR, Lough RG (2005) A synthesis of large scale patterns in the planktonic prey of larval and juvenile cod. *ICES CM 2005/AA:10*, 1–19.

Hilborn R, Walters CJ (1992) *Quantitative Fish Stock Assessment - Choice, Dynamics and Uncertainty*. Kluwer Academic Publishers.

Houde ED (1997) Patterns and trends in larval-stage growth and mortality of teleost fish. *Journal of Fish Biology*, **51**, 52–83.

Houde ED (2008) Emerging from Hjort's Shadow. *Journal of Northwest Atlantic Fishery Science*, **41**, 53–70.

Hurst TP, Fernandez ER, Mathis JT, Miller JA, Stinson CM, Ahgeak EF (2012) Resiliency of juvenile walleye pollock to projected levels of ocean acidification. *Aquatic Biology*, **17**, 247–259.

Hurst TP, Fernandez ER, Mathis JT (2013) Effects of ocean acidification on hatch size and larval growth of walleye pollock (*Theragra chalcogramma*). *ICES Journal of Marine Science*, **70**, 812–822.

Hurst TP, Laurel BJ, Mathis JT, Tobosa LR (2015) Effects of elevated CO_2 levels on eggs and larvae of a North Pacific flatfish. *ICES Journal of Marine Science*, **71**, 982–990.

ICES (2014a) *Report of the Baltic Fisheries Assessment Working Group (WGBFAS), 3-10 April 2014*. ICES HQ, Copenhagen, Denmark, 919 pp..

ICES (2014b) *Report of the Arctic Fisheries Working Group (AFWG), 2014, Lisbon, Portugal*. 656 pp..

ICES (2017) *ICES Advice on fishing opportunities, catch, and effort cod. 27.1-2*.

IPCC (2007) *Climate change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Solomon S, Qin D, Manning M, Marquis M, Averyt K, Tignor M, LeRoy Miller H, Chen Z). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 996 pp..

IPCC (2013) *Climate Change 2013 - The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel of Climate Change* (eds Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, Cambridge, 1535 pp..

Ishimatsu A, Hayashi M, Kikkawa T (2008) Fishes in high-CO_2, acidified oceans. *Marine Ecology Progress Series*, **373**, 295–302.

Kaltin S, Anderson LG, Olsson K, Fransson A, Chierici M (2002) Uptake of atmospheric carbon dioxide in the Barents Sea. **38**, 31–45.

Kjørsvik E, Olsen C, Wold PA, Hoehne-Reitan K, Cahu CL, Rainuzzo J, Olsen AI, Øie G, Olsen Y (2009) Comparison of dietary phospholipids and neutral lipids on skeletal development and fatty acid composition in Atlantic cod (*Gadus morhua*). *Aquaculture*, **294**, 246–255.

Kjesbu OS, Bogstad B, Devine JA, Gjøsæter H, Howell D, Ingvaldsen RB, Nash RDM, Skæraasen JE (2014) Synergies between climate and management for Atlantic cod fisheries at high latitudes. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 3478–83.

Königstein S, Dahlke FT, Stiasny MH, Storch D, Clemmesen C, Pörtner H-O (2017) Forecasting future recruitment success for Atlantic cod in the warming and acidifying Barents Sea. *Global Change Biology*.

Kroeker KJ, Kordas RL, Crim R, Hendriks IE, Ramajo L, Singh GS, Duarte CM, Gattuso J-P (2013) Impacts of ocean acidification on marine organisms: Quantifying sensitivities and interaction with warming. *Global Change Biology*, **19**, 1884–

1896.

Lall SP, Lewis-McCrea LM (2007) Role of nutrients in skeletal metabolism and pathology in fish - An overview. *Aquaculture*, **267**, 3–19.

Lam VWY, Cheung WWL, Sumaila UR (2014) Marine capture fisheries in the Arctic: winners or losers under climate change and ocean acidification? *Fish and Fisheries*, 1–23.

Lefevre S, McKenzie DJ, Nilsson GE (2017) Models projecting the fate of fish populations under climate change need to be based on valid physiological mechanisms. *Global Change Biology*, 1–11.

Llopiz J, Cowen R, Hauff M, Ji R, Munday PL, Muhling BA, Peck MA, Richardson DE, Sogard S, Sponaugle S (2014) Early Life History and Fisheries Oceanography: New Questions in a Changing World. *Oceanography*, **27**, 26–41.

MacNeil MA, Graham NAJ, Cinner JE, Dulvy NK, Loring PA, Jennings S, Polunin NVC, Fisk AT, McClanahan TR (2010) Transitional states in marine fisheries: adapting to predicted global change. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **365**, 3753–63.

Malzahn AM, Aberle N, Clemmesen C, Boersma M (2007) Nutrient limitation of primary producers affects planktivorous fish condition. *Limnology and Oceanography*, **52**, 2062–2071.

Maneja RH, Frommel AY, Browman HI, Clemmesen C, Geffen AJ, Folkvord A, Piatkowski U, Durif CMF, Bjelland R, Skiftesvik AB (2013) The swimming kinematics of larval Atlantic cod, *Gadus morhua* L., are resilient to elevated seawater $p\mathrm{CO}_2$. *Marine Biology*, **160**, 1963–1972.

Maneja R, Frommel A, Geffen A, Folkvord A, Piatkowski U, Chang M, Clemmesen C (2013) Effects of ocean acidification on the calcification of otoliths of larval Atlantic cod *Gadus morhua*. *Marine Ecology Progress Series*, **477**, 251–258.

Meekan MG, Fortier L (1996) Selection for fast growth during the larval life of Atlantic cod *Gadus morhua* on the Scotian Shelf. *Marine Ecology Progress Series*, **137**, 25–37.

van der Meeren T, Mangor-Jensen A, Pickova J (2007) The effect of green water and light intensity on survival, growth and lipid composition in Atlantic cod (*Gadus morhua*) during intensive larval rearing. *Aquaculture*, **265**, 206–217.

Mehrbach C, Culberson CH, Hawley JE, Pytkowicz RM (1973) Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnology and Oceanography*, **18**, 897–907.

Melzner F, Gutowska M a., Langenbuch M, Dupont S, Lucassen M, Thorndyke MC, Bleich M, Pörtner H-O (2009a) Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny?

Biogeosciences Discussions, **6**, 4693–4738.

Melzner F, Göbel S, Langenbuch M, Gutowska MA, Pörtner HO, Lucassen M (2009b) Swimming performance in Atlantic Cod (*Gadus morhua*) following long-term (4-12 months) acclimation to elevated seawater $p\mathrm{CO}_2$. *Aquatic Toxicology*, **92**, 30–37.

Melzner F, Thomsen J, Koeve W, Oschlies A, Gutowska A, Bange HW, Hansen HP, Körtzinger A (2012) Future ocean acidification will be amplified by hypoxia in coastal habitats. *Marine Biology*, **160**, 1875–1888.

Miller GM, Watson S-A, Donelson JM, McCormick MI, Munday PL (2012) Parental environment mediates impacts of increased carbon dioxide on a coral reef fish. *Nature Climate Change*, **2**, 858–861.

Munday PL, Dixson DL, Donelson JM, Jones GP, Pratchett MS, Devitsina G V., Døving KB (2009a) Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 1848–52.

Munday PL, Donelson JM, Dixson DL, Endo GGK (2009b) Effects of ocean acidification on the early life history of a tropical marine fish. *Proceedings of The Royal Society B: Biological sciences*, **276**, 3275–83.

Munday PL, Dixson DL, McCormick MI, Meekan M, Ferrari MCO, Chivers DP (2010) Replenishment of fish populations is threatened by ocean acidification. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 12930–4.

Munday PL, Gagliano M, Donelson J, Dixson D, Thorrold S (2011) Ocean acidification does not affect the early life history development of a tropical marine fish. *Marine Ecology Progress Series*, **423**, 211–221.

Munday PL, Watson S-A, Parsons DM, King A, Barr NG, Mcleod IM, Allan BJM, Pether SMJ (2015) Effects of elevated CO_2 on early life history development of the yellowtail kingfish, *Seriola lalandi*, a large pelagic fish. *ICES Journal of Marine Science,* **73** (3), 641-649.

Nissling A, Westin L (1997) Salinity requirements for successful spawning of Baltic and Belt Sea cod and the potential for cod stock interactions in the Baltic Sea. *Marine Ecology Progress Series*, **152**, 261–271.

Omstedt A, Edman M, Claremar B, Frodin P, Gustafsson E, Humborg C, Hägg H, Mörth M, Rutgersson A, Schurgers G, Smith B, Wällstedt T, Yurova A (2012) Future changes in the Baltic Sea acid-base (pH) and oxygen balances. *Tellus B*, **64**.

Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RA, Gnanadesikan A, Gruber N, Ishida A, Joos F, Key RM, Lindsay K, Maier-Reimer E, Mataer R, Monfray P, Mouchet A, Najjar RG, Plattner G-K, Rodgers KB, Sabine CL, Sarmiento JL, Schlitzer R, Slater RD, Totterdell IJ, Weirig M-F, Yamanaka Y, Yool A (2005)

Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*, **437**, 681–686.

Ottersen G, Bogstad B, Yaragina NA, Stige LC, Vikebo FB, Dalpadado P (2014) A review of early life history dynamics of Barents Sea cod (*Gadus morhua*). *ICES Journal of Marine Science*, **71**, 2064–2087.

Pauly D, Christensen V, Guénette S, Pitcher TJ, Sumaila R, Walters CJ, Watson R, Zeller D (2002) Towards sustainability in world fisheries. *Nature*, **418**, 689–695.

Le Pecq JB, Paoletti C (1966) A new fluorometric method for RNA and DNA determination. *Annual Review of Biochemistry*, **17**, 100–107.

Perry SF, Gilmour KM (2006) Acid – base balance and CO_2 excretion in fish : Unanswered questions and emerging models. *Respiratory Physiology and Neurobiology*, **154**, 199–215.

Perry AL, Low PJ, Ellis JR, Reynolds JD (2005) Climate change and distribution shifts in marine fishes. *Science*, **308**, 1912–1915.

Perry DM, Redman DH, Widman JC, Meseck S, King A, Pereira JJ (2015) Effect of ocean acidification on growth and otolith condition of juvenile scup, *Stenotomus chrysops*. *Ecology and Evolution*, **5**, 4187–4196.

Pierrot D, Lewis E, Wallace DWR (2006) MS Excel Program developed for CO_2 system calculations, ORNL/CDIAC-105. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee, U.S.

Pimentel MS, Faleiro F, Dionisio G, Repolho T, Pousao-Ferreira P, Machado J, Rosa R (2014) Defective skeletogenesis and oversized otoliths in fish early stages in a changing ocean. *The Journal of Experimental Biolology*, **217**, 2062–2070.

Pinsky ML, Jensen OP, Ricard D, Palumbi SR (2011) Unexpected patterns of fisheries collapse in the world's oceans. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 8317–8322.

Poloczanska ES, Brown CJ, Sydeman WJ, Kiessling W, Schoeman DS, Moore PJ, Brander K, Bruno JF, Buckley LB, Burrows MT, Duarte CM, Halpern BS, Holding J, Kappel CV, O'Conner MI, Pandolfi JM, Parmesan C, Schwing F, Thompson SA, Richardson AJ (2013) Global imprint of climate change on marine life. *Nature Climate Change*, **3**, 919–925.

Pörtner H-O (2010) Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *The Journal of Experimental Biology*, **213**, 881–893.

Puvanendran V, Brown JA (1999) Foraging, growth and survival of Atlantic cod larvae reared in different prey concentrations. *Aquaculture*, **175**, 77–92.

Le Quesne WJF, Pinnegar JK (2012) The potential impacts of ocean acidification: scaling from physiology to fisheries. *Fish and Fisheries*, **13**, 333–344.

Quinn TJ, Deriso RB (1999) *Quantitative Fish Dynamics*. Oxford University Press.

Réveillac E, Lacoue-Labarthe T, Oberhänsli F, Teyssié JL, Jeffree R, Gattuso JP, Martin S (2015) Ocean acidification reshapes the otolith-body allometry of growth in juvenile sea bream. *Journal of Experimental Marine Biology and Ecology*, **463**, 87–94.

Ricker WE (1954) Stock and Recruitment. *Journal of the Fisheries Research Board of Canada*, **11**, 559–623.

Riebesell U, Fabry VJ, Hansson L, Gattuso J-P (2010) *Guide to Best Practices in Ocean Acidification Research and Data Reporting*. 260 pp.

Rummer JL, Munday PL (2016) Climate change and the evolution of reef fishes: Past and future. *Fish and Fisheries*, 22–39.

Sabine CL, Feely RA, Gruber N, Key RM, Lee K, Bullister JL, Wanninkhof R, Wong CS, Wallace DWR, Tilbrook B, Millero FJ, Peng, T-H, Kozyr A, Ono T, Rios AF (2004) The oceanic sink for anthropogenic CO_2. *Science*, **305**, 367–71.

Schade FM, Clemmesen C, Wegner KM (2014) Within- and transgenerational effects of ocean acidification on life history of marine three-spined stickleback (*Gasterosteus aculeatus*). *Marine Biology*, **161**, 1667–1676.

Schnell NK, Konstantinidis P, Johnson GD (2016) High-proof Ethanol Fixation of Larval and Juvenile Fishes for Clearing and Double Staining. *Copeia*, **104**, 617–622.

Scripps Institution of Oceanography, The Keeling Curve. accessible: https://scripps.ucsd.edu/programs/keeligcurve/, accessed: 18th July 2017

Seljeset O, Vollset KW, Folkvord A, Geffen AJ (2010) The role of prey concentration and size range in the growth and survival of larval cod. *Marine Biology Research*, **6**, 251–262.

Shama LNS, Wegner KM (2014) Grandparental effects in marine sticklebacks: transgenerational plasticity across multiple generations. *Journal of Evolutionary Biology*, **27**, 2297–2307.

Shama LNS, Mark FC, Strobel A, Lokmer A, John U, Wegner KM (2016) Transgenerational effects persist down the maternal line in marine sticklebacks: gene expression matches physiology in a warming ocean. *Evolutionary Applications*, **9**, 1096–1111.

Silva CSE, Novais SC, Lemos MFL, Mendes S, Oliveira AP, Gonçalves EJ, Faria AM (2016) Effects of ocean acidification on the swimming ability, development and biochemical responses of sand smelt larvae. *Science of The Total Environment*, **563–564**, 89–98.

Steinacher M, Joos F, Frölicher TL, Plattner G-K, Doney SC (2008) Imminent ocean acidification projected with the NCAR global coupled carbon cycle-climate model. *Biogeosciences Discussions*, **5**, 4353–4393.

Stiasny MH, Mittermayer FH, Sswat M, Voss R, Jutfelt F, Chierici M, Puvanendran V, Mortensen A, Reusch TBH, Clemmesen C (2016) Ocean Acidification Effects on Atlantic Cod Larval Survival and Recruitment to the Fished Population. *PLOS ONE*, **11**, e0155448.

Sunday JM, Calosi P, Dupont S, Munday PL, Stillman JH, Reusch TBH (2014) Evolution in an acidifying ocean. *Trends in Ecology & Evolution*, **29**, 117–125.

Sundby S, Nakken O (2008) Spatial shifts in spawning habitats of Arcto-Norwegian cod induced by climate change. *ICES Journal of Marine Science*, **65**, 953–962.

Walker M, Kimmel C (2007) A two-color acid-free cartilage and bone stain for zebrafish larvae. *Biotechnic & Histochemistry*, **82**, 23–28.

Welch MJ, Watson S, Welsh JQ, McCormick MI, Munday PL (2014) Effects of elevated CO_2 on fish behaviour undiminished by transgenerational acclimation. *Nature Climate Change*, **4**, 1086–1089.

7 DANKSAGUNG

Thorsten, thank you for pushing me to always aim a little higher and all your input to the work and to my career planning in general.

Martin, thank you for giving me a new professional home in your group, for supporting the project throughout and for giving me the opportunity to venture to new scientific horizons.

Catriona, I will never quite find the right words to describe what you have and continue to mean to me, so I have to trust that you know. I cannot possibly list all the things that I am grateful for and that you have done for me. Without you, your constant support and belief in me, I wouldn't be the scientist or the person I am today. One person, who has your back, no matter what, is all you need... I very much hope that his is not the end of the road for us.

Rudi, you appeared on the scene, when I only had a vague idea of where I wanted to go with this project and I am eternally grateful that you did and that it turned into everything that it has. It's such pleasure to work with you. Thank you for your many kind words of encouragement!

Micha, also known as "the Boss", you were the best mentor, friend, honorary big brother and colleague, I could have asked for during my first large-scale experiments and I still treasure your friendship immensely. Your compliments during the last days of this endeavor came unexpected, but they haven't missed! None of this would have happened without you, boss!

Jörn, thank you for every discussion about fatty livers and our minutes of fame at the UN, every talk over beer on the roof top terraces of Manhattan and of course also for the continued help with funding for ICES ASCs (but let's forget about that!).

Christian, Felix, Melanie, Sophie, Miguel, Julia, Katrin, Julia, Kira, Jörn and Rudi thank you for sharing a whole bunch of offices through the years. I enjoyed my time in each one and you made this process way more fun!

Fredrik, Flemming, Elettra, Felix, Gwen, Julia, Chris, Johanna and every BIOAcid scientist for sharing some really great times during the experiments and A LOT of physical and emotional and mental support during many, many night shifts and a lot of hard work.

Fredrik, thank you for the amazing pictures of our beautiful little larvae.

The whole KOSMOS Consortium and **all scientists and staff at the Sven Lovén Centre in Kristineberg and the Centre for Marine Aquaculture in Tromsø** for all your support during all experiments and a lot of fun and great times along the way.

To our student assistants Luisa, Ramona, Serra and Julia for your support measuring all my larvae and everything else you've done.

Everyone in the EV at the GEOMAR and everyone in EREE at the University, as well as everyone else I may have forgotten during this period of faulty 'PhD brain'.

Tausend Dank gehen an **Merle**, die beste Mitbewohnerin und beste, beste Freundin, die sich eine Doktorandin wünschen kann. Ohne dich, den Kaffee ans Bett jeden Morgen, viele, viele Gläser Wein auf dem Balkon (hin und wieder auch Ramazzotti, wenn es akut wurde) und viele Booty-Shakes zu Meaghan Trainor, hätte meine mentale Verfassung deutlich mehr gelitten.

Mama, vor allem anderen, danke ich dir dafür, dass du meinen eigenen Träumen, Wünschen und Vorstellungen nie gedankliche Grenzen gesetzt hast. Du hast mir nie das Gefühl gegeben, dass ich zu hoch träume oder irgendetwas nicht erreichen könnte oder als wärst du je überrascht gewesen, wenn es geklappt hat, egal ob es um St Andrews, Brüssel oder New York ging. Jede junge Frau braucht eine Mutter wie dich! You are one of my giants. Ich sitze jederzeit gerne wieder in Oslo am Hafen mit dir und stoße auf eine Einladung zu den United Nations an!

Mein Vater, ohne dich wäre mein Studium nicht möglich geworden. Ich hoffe, du bist stolz auf das Ergebnis!

Peter und Jan, Wenige haben das Glück zwei wundervolle Brüder zu haben. Ich bin unglaublich froh, dass es euch gibt. Slàinte!

Svenja, du bist die älteste Freundin, die ich habe. Danke, dass ich bei dir immer noch ein Stück Zuhause finde. Deine Freundschaft bedeutet mir unglaublich viel. Bei dir habe ich das Gefühl, ich habe doch eine Schwester.

Zu Allerletzt danke ich **Hanneliese Folkers**. Oma, ich schaffe immer noch kein ganzes Stück Torte, daher muss ein Doktortitel für heute reichen. Du warst mein Fixpunkt, mein Moment der Stille und der Stabilität und dafür bin ich dir für immer dankbar. Ich glaube, du wusstest ganz genau, wann du los lassen konntest. Svenja und ich haben alles im Griff, keine Sorge! Trotzdem fehlst du.

8 EIDESSTATTLICHE ERKLÄRUNG

Eidesstattliche Erklärung

Hiermit bestätige ich, dass die vorliegende Arbeit mit dem Titel:

Effects of Ocean Acidification on Atlantic Cod Larvae
(*Gadus morhua*)

Von mir selbstständig verfasst worden ist und keine weiteren Quellen und Hilfsmittel als die angegebenen verwendet wurden.

Die vorliegende Arbeit ist unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft entstanden und wurde weder im Rahmen eines Prüfungsverfahrens an anderer Stelle vorgelegt noch veröffentlicht.

Veröffentlichte oder zur Veröffentlichung eingereichte Manuskripte wurden kenntlich gemacht.

Ich erkläre mich einverstanden, dass diese Arbeit an die Bibliothek des GEOMAR Helmholtz Zentrum für Ozeanforschung Kiel und die Universitätsbibliothek der Christian-Albrechts-Universität zu Kiel weitergeleitet wird.

Kiel, August 2017

Martina H. Stiasny

9 CURRICULUM VITAE

Curriculum Vitae

Martina H. Stiasny

Date of birth	30th May 1989
Place of birth	Jever, Germany
Nationality	German

EDUCATION

Since 2014	**Group of Evolutionary Ecology of Marine Fishes, GEOMAR Helmholtz Centre for Ocean Research, Kiel Germany and Group of Environmental, Resource and Ecological Economics, Christian-Albrechts-Universität zu Kiel, Germany** *PhD Student, Thesis entitled „Ocean acidification effects on Atlantic cod“*
March to July 2016	**Executive Agency for Small and Medium-sized Enterprises, Unit A3 (Implementation of the European Maritime and Fisheries Fund), EU Commission, Brussels, Belgium** *Blue Book Trainee in the official European Commission Traineeship Program*
March 2014	**GEOMAR Helmholtz Centre for Ocean Research, Kiel, Germany** *Master of Science in Biological Oceanography (final grade:1.3)* *Master thesis: The effect of ocean acidification and warming on survival and growth of Atlantic cod (Gadus morhua) larvae (grade:1.1)*
November 2011	**University of St Andrews, St Andrews, Scotland, UK** *Bachelor of Science (Biology) with distinction*
2010	**Sea Education Association (SEA), Woods Hole, MA, USA and RV R.C. Seaman** *Summer Semester, Class S-230, Oceanography, Nautical Science and Maritime Studies; Research Project: Epipelagic Nekton between Hawai'i and San Francisco and its role in the food web*
2008	**St Joseph-Gymnasium, Rheinbach, Germany** *German Abitur (final grade: 1.6)*
Summer Semester 2007	***Fördern-Fordern-Forschen Program, University Bonn, Germany*** Program for highly motivated and talented pupils to study at university level while still at school, one semester of Paleontology completed
2005-2006	***Carleton Jones High School, Carletonville, South Africa*** Year abroad at an English-speaking High School

RESEARCH AND WORK EXPERIENCE

04-05/2017	**Research Stay at the Centre for Ecological and Evolutionary Synthesis (CEES), University of Oslo, Norway**
09-12/2016	**Executive Agency for Small and Medium-sized Enterprises, Unit A3 (Implementation of the European Maritime and Fisheries Fund), EU Commission, Brussels, Belgium** *Project Officer*
10/2014 – 09/2015	**GEOMAR, Kiel and Landesamt für Umwelt, Naturschutz und Geologie Mecklenburg-Vorpommern, Germany** *Research Associate*
2011 - 2012	**Zoological Museum, Kiel** *Education assistant*
2009 - 2011	**Member of the Oxford University Press Student Panel for the Biosciences**
June to July 2011	**GEOMAR, Kiel, Germany** *Internship with Dr. Rainer Froese in the research group 'Evolutionary Ecology of Marine Fishes' and fishbase.org*
August 2007	**IfM-GEOMAR, Kiel, Germany,** *Summer School of Marine Geosciences* Special Research Unit '*Fluids and Volatiles in the Subduction Zone'*

SKILLS

Languages	**German** (native), **English** (fluent), **French** (advanced), **Russian** (beginner)
IT Skills	excellent MS Office skills, R (advanced), MatLab and SPSS (basic skills)

PARTICIPATION

Workshop on Understanding the Impacts and Consequences of Ocean Acidification for Commercial Species and End-users (**WKACIDUSE**) of the **International Council for the Exploration of the Sea**, December 2016, ICES Headquarters, Copenhagen, Denmark

Moscow Summer Academy on Economic Growth and Governance of Natural Resources, Moscow State University, Moscow, Russia, July 2015

SCIENTIFIC PUBLICATIONS

Königstein, S., Dahlke, F.T., **Stiasny, M.H.,** Storch, D., Clemmesen, C., Pörtner, H.-O. (2017) Forecasting future recruitment success for Atlantic cod in the warming and acidifying Barents Sea, Global Change Biology

Stiasny, M.H., Mittermayer, F.H., Sswat, M., Voss, R., Jutfelt, F., Chierici, M., Puvanendran, V., Mortensen, A., Reusch, T.B.H., Clemmesen, C. (2016) Ocean acidification effects on Atlantic cod larval survival and recruitment to the fished population, PLoS ONE, 11 (8)

Bach, L.T. *et al.* (2016) Influence of ocean acidification on a natural winter-to-summer plankton succession: First insights from a long-term mesocosm study draw attention to periods of low nutrient concentrations, PLoS ONE, 11 (8)

Stiasny, M.H., Mittermayer, F.H., Göttler, G., Bridges, C.R., Falk-Petersen, I.-B., Puvanendran, V., Mortensen, A., Reusch, T.B.H., Clemmesen, C. (submitted to Scientific Reports) Parental acclimation effects in response to ocean acidification in Atlantic cod

Voss, R., Quaas, M.F., **Stiasny, M.H.**, Hänsel, M., Stecher Justiniano Pinto, G., Lehmann, A., Reusch, T.B.H., Schmidt, J.O. (submitted to Journal of Applied Ecology) Economic viability of Baltic cod fishery requires both – meeting global climate targets and improving local ecosystem management

Sswat, M., **Stiasny, M.H.,** Jutfelt, F., Riebesell, U., Clemmesen, C. (submitted to PLoS ONE) Performance and survival of larval Atlantic herring under the combined effects of elevated temperatures and ocean acidification

Sswat, M., **Stiasny, M.H.,** Algueró-Muñiz, M., Jutfelt, F., Clemmesen, C., Riebesell, U., (submitted to PNAS), Indirect effects of Ocean Acidification on growth and survival of herring larvae from a large scale mesocosm experiment

SCIENTIFIC PRESENTATIONS

Stiasny, M.H., "Do we have to take the bitter with the sour" – A next generation scientist's consideration of risk of ocean acidification on fish and fisheries and how these can be minimized, United Nations' Ocean Conference, Side Event 'Ocean Acidification – What can we do about it?', United Nations' Headquarters, New York City, NY, USA, June 2017

Stiasny, M.H., Mittermayer, F.H., Sswat, M., Hänsel, M., Voss, R., Quaas, M., Reusch, T., Clemmesen, C., Cod and CO_2- So what? Climate Change Effects on Atlantic Cod Recruitment and Fisheries, Early Career Scientists Conference, International Council for the Exploration of the Sea (ICES) and North Pacific Marine Science Organization (PICES), Busan, South Korea, May/June 2017

Stiasny, M.H., Sswat, M., Mittermayer, F.H., Hänsel, M., Voss, R., Reusch, T.B.H., Quaas, M., Clemmesen, C., Climate Change impacts on Atlantic cod, European Commission, DG Mare Lunchtime Conference Series, October 2016

Stiasny, M.H., Hänsel, M., Clemmesen, C., Dahlke, F., Mittermayer, F.H., Quaas, M., Reusch, T., Storch, D., Voss, R, Socio-economic impacts of ocean acidification and warming on Barents Sea Cod, International Council for the Exploration of the Sea, Annual Science Conference, Riga, September 2016

Clemmesen, C., **Stiasny, M.H.,** et al., Effects of Ocean Acidification on Atlantic cod larvae of different populations in terms of survival and carry over of early life for recruitment to the fished stock, 40th Annual Larval Fish Conference, Solomons, USA, 2016

Stiasny, M.H., Sswat, M., Mittermayer, F.H., Voss, R., Jutfelt, F., Chierici, M., Puvanendran, V., Mortensen, A., Reusch, T.B.H., Clemmesen, C. Effects of end-of-the-century ocean acidification on Atlantic cod larvae of different populations in terms of survival, growth and recruitment to the fished stocks. International Council for the Exploration of the Sea, Annual Science Conference, Copenhagen, September 2015

Clemmesen, C., Maneja, R., **Stiasny, M.H.,** Frommel, A., Folkvord, A., Piatkowski, U., Geffen, A., Effects of Ocean Acidification on otolith growth and size selective mortality of larval Atlantic cod (*Gadus morhua*) and herring (*Clupea harengus*), CEFAS, Lowestoft, October 2014

Clemmesen, C., **Stiasny, M.H.**, Sswat, M. Ocean acidification and warming affect survival and growth of Atlantic cod (*Gadus morhua*) larvae. Larval fish conference, Quebec, Canada. August 2014

Kiel, August 2017