Direct and indirect effects of ocean acidification on early life stages of Atlantic cod and herring

Dissertation

zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften

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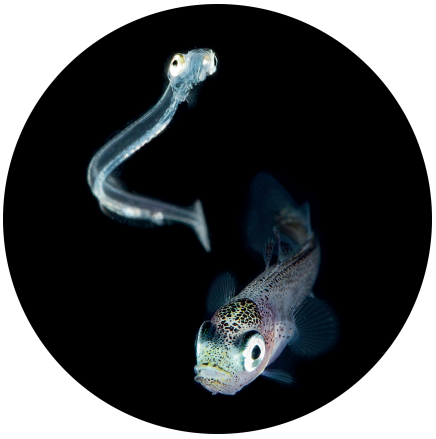

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Summary

Since fish are of major economical importance and hold crucial ecological positions in the marine food web, concerns are arising of how fish will be affected in the future, as a consequence of rising atmospheric CO_2 concentrations. Rising concentrations of this potent greenhouse gas, in the atmosphere, causes ocean warming. At the same time, CO_2 in the ocean reacts with seawater forming carbonic acid, resulting in ocean acidification. CO_2 levels are projected to reach $\sim$800 $\mu\mathrm{atm}$ $p\mathrm{CO}_2$ by the year 2100, compared to today's $\sim$400 $\mu\mathrm{atm}$. As a consequence, the ocean is estimated to become warmer by at least 1.5$^{\circ}\mathrm{C}$ and more acidic, illustrated by a drop in pH of $\sim$0.4 units. Fish larvae are considered highly susceptible to the direct impact of ocean acidification, since gills for effective acid base regulation are not yet developed. This direct physiological effect of ocean acidification on fish larvae was assessed for multiple traits, such as growth, development and behavior, revealing variable sensitivities to elevated CO_2 concentrations between species. However, only a few studies directly investigated the effect of ocean acidification on fish larval survival, a critical bottleneck for recruitment in fish populations. Furthermore, most studies have so far investigated the effects of ocean acidification at *ad libitum*, i.e. unlimited food conditions. Under *ad libitum* food conditions increased energy demand for processes, such as metabolism and acid base regulation, may be readily met and, thus, mask potential negative effects of ocean acidification. In general when food supply is limited, larvae experience major drawbacks in growth performance and survival. Energy demand may be further intensified, when elevated temperatures increase metabolic rates, and thus, decrease the amount of energy available for other processes, such as acid base regulation. This in turn could increase the risk of negative ocean acidification effects. One of the most important drivers of survival and recruitment in fish larvae is the availability of suitable prey organisms. Changes in prey availability and fish recruitment have already been attributed to ocean warming. Ocean acidification can also be expected to alter marine food webs in multiple ways, as it could impair calcifying organisms, whereas photosynthesis of primary producers and, thus, production may increase. Ocean acidification induced changes in the food web and concomitant availability of prey organisms may indirectly affect fish larval growth performance and survival. The combined direct physiological and indirect food web effect of ocean acidification on fish larvae has not been tested so far, as it requires a simultaneous treatment of fish larvae and their food web with CO_2. This requires (1) the enclosure of a sufficient number of fish larvae in their natural food web and (2) a sufficient temporal duration, so ocean acidification effects on certain groups of the food web can manifest and trigger changes in the community composition. Mesocosms can fulfil these requirements, but so far mesocosm studies on the effects of ocean acidification on plankton communities, excluded fish larvae.

This dissertation is aimed to investigate the direct physiological, and indirect food web effects of ocean acidification on fish larvae of two commercially important species of the North Atlantic, herring and cod. The first study, performed in the lab, tested the combined effect of end-of-century CO_2 concentrations, and elevated temperature, on herring larval performance. Herring larval growth, and survival, was found to decrease in the warmer temperature treatment, which was, most likely, caused by an increased energy demand limited by food supply, since both tested temperatures are within the optimal thermal range of herring. Although energy supply was low, no substantial effect of CO_2 on herring larval growth performance and survival was found. Thus herring larvae can be considered tolerant to the direct effects of end-of-century CO_2 concentrations. The direct effect of the same CO_2-concentration was tested in two other lab-based experiments on Atlantic cod larvae originating from two distinct populations, the Western Baltic and the Barents Sea. Cod larvae from both populations showed a substantial decrease in survival under end-of-century CO_2 concentrations. Based on these results a massive decrease in recruitment of both populations to 8 and 24 % of today's recruitment was projected. The last study, performed in mesocosms, combined direct physiological and indirect food web effects of ocean acidification. Survival and growth performance of herring larvae were tested within a simulated future ocean food web. The mesocosms enclosed a natural plankton community, with half of the mesocosms set to CO_2-concentrations close to the projection for the end-of-century, while the other half was kept as untreated controls. Herring larvae hatched in these environments and exclusively fed on the enclosed prey organisms. Larval survival was increased under elevated CO_2 compared to ambient conditions. This effect was related to increased prey abundances from an ocean acidification-induced boost in phytoplankton primary production and abundance. However, this positive indirect effect of ocean acidification via the food web did no increase larval growth rates. Higher larval abundances in the elevated CO_2 treatment may have executed an increased predation pressure, resulting in lower prey availability per larvae. Thus, increased competition may have prevented a positive food web effect of ocean acidification on herring larval growth. The results of these studies illustrate how the direct physiological effects of projected ocean acidification may differ between CO_2-tolerant and -sensitive species and how fish larvae may be indirectly affected by ocean acidification.

In conclusion, CO_2-tolerant fish larvae such as herring can be expected to benefit when ocean acidification increases prey availability. However, performance of these CO_2-tolerant fish larvae may equally be negatively affected when ocean acidification decreases prey availability. Under the scenario of ocean acidification-induced food limitation, CO_2-sensitive fish larvae such as Atlantic cod can be expected to show a persistence or intensification of direct negative effects of ocean acidification.

One interesting follow-up question resulting from this dissertation is, if an ocean acidification-induced increase in prey abundances can mitigate, or even outplay, negative ocean acidification effects in sensitive fish larvae. Therefore, future research needs to assess under which circumstances ocean acidification increases and decreases prey availability for fish larvae. Furthermore, the relative importance of direct and indirect effects of ocean acidification on fish larvae needs to be investigated, especially when energy demand is increased under the effects of ocean warming and ocean acidification.

Zusammenfassung

Fisch ist von großer, ökonomischer Bedeutung und gleichzeitig ein essentieller Bestandteile des marinen Nahrungsnetzes. Die Auswirkungen der stetig steigenden CO_2-Konzetration in der Atmosphäre auf die weltweiten Fischbestände sind jedoch weitgehend unbekannt. CO_2 ist eines der bedeutendsten Treibhausgase, welches zur Erwärmung der Erde und somit auch der Ozeane beiträgt. Gleichzeitig löst sich CO_2 in den Ozeanen und reagiert mit Wasser zu Kohlensäure, was die Versauerung der Ozeane zur Folge hat. Wissenschaftliche Prognosen besagen, dass bis zum Jahr 2100 mit einem Anstieg der atmosphärischen CO_2-Konzentration von derzeit $\sim$400 $\mu\mathrm{atm}$ $p\mathrm{CO}_2$ auf $\sim$800 $\mu\mathrm{atm}$ gerechnet werden kann. Dies hätte eine Erwärmung um mindestens 1.5 $^{\circ}\mathrm{C}$, sowie eine Versauerung der Ozeane um $\sim$0.4 pH Einheiten zur Folge. Fischlarven reagieren in der Regel besonders anfällig auf Ozeanversauerung, da sie noch keine Kiemen entwickelt haben und dadurch ihren Säure-Base Haushalt nur eingeschränkt regulieren können. Bei Untersuchungen zum direkten physiologischen Effekt von Ozeanversauerung auf Merkmale wie z.B. Wachstum, Entwicklung und Verhalten von Fischlarven, zeigen verschiedene Arten eine unterschiedliche Anfälligkeit für erhöhte CO_2-Konzentrationen. Allerdings untersuchten bisher nur einige wenige Studien dabei den direkten Effekt von Ozeanversauerung auf das Überleben von Fischlarven, welches eine kritische Phase für die Nachwuchsproduktion und somit Bestandsentwicklung von Fischen darstellt.

Da die Verfügbarkeit von Nahrungsorganismen in diesen Studien selten ein limitierender Faktor war, könnte es sein, dass potenzielle negative Effekte von Ozeanversauerung auf Fischlarven nicht entdeckt wurden. Generell sind bei Futtermangel Wachstumseinbußen und verminderte Überlebenseraten bei Fischlarven zu erwarten. Bei verminderter Nahrungsverfügbarkeit steht potenziell ebenfalls weniger Energie für andere Prozesse wie z.B. die Regulierung des Säure-Base-Haushalts zur Verfügung, wodurch negative Effekte von Ozeanversauerung zu Tage treten könnten. Dieser Effekt könnte durch erhöhte Temperaturen noch verstärkt werden, da letztere den Metabolismus ankurbeln und den Energiebedarf weiter erhöhen. Veränderte Nahrungsbedingung und somit Bestandsentwicklungen von Fischen wurden bereits mit der Erwärmung der Ozeane in Verbindung gebracht. Auch Ozeanversauerung könnte das Nahrungsnetz in den Meeren stark beeinflussen, da zum einen kalzifizierende Organismen durch Ozeanversauerung beeinträchtigt werden können, aber gleichzeitig ein Anstieg der Primärproduktion durch verstärkte Photosynthese möglich ist. Somit könnte Ozeanversauerung durch Veränderungen im Nahrungsnetz, und daraus resultierenden Schwankungen in der Verfügbarkeit von Futterorganismen, das Überleben von Fischlarven indirekt beeinflussen. Bisher wurde die Interaktion von direkten, physiologischen und indirekten, nahrungsabhängigen Effekten von Ozeanversauerung auf Fischlarven noch nicht untersucht. Hierfür ist eine simultane Exposition von Fischlarven

und ihrem Nahrungsnetz gegenüber erhöhtem CO_2 notwendig. Dies erfordert (1) die Inkubation einer ausreichenden Anzahl von Fischlarven in ihrem natürlichen Nahrungsnetz und (2) eine genügend lange Laufzeit, damit sich potenzielle Effekte von Ozeanversauerung in einzelnen Gruppen der Planktongemeinschaft manifestieren und Veränderungen im Nahrungsnetz auslösen können. Mesokosmen erfüllen diese Bedingungen, jedoch wurden Fischlarven bei bisherigen Studien zum Einfluss von Ozeanversauerung auf Planktongemeinschaften nicht berücksichtigt.

Diese Dissertation hat sich zum Ziel gesetzt, die direkten physiologischen Effekte sowie die indirekten nahrungsabhängigen Effekte von Ozeanversauerung auf Fischlarven von zwei kommerziell wichtigen Arten des Nord-Atlantiks zu untersuchen: Hering und Dorsch. In der ersten Laborstudie wurde zunächst untersucht wie Heringslarven direkt von erhöhter CO_2-Konzentration (prognostiziert für das Jahr 2100) und erhöhter Temperatur beeinflusst werden. Es stellte sich heraus, dass das Wachstum und Überleben der Heringslarven bei erhöhter Wassertemperatur stärker beeinträchtigt war. Dies ist vermutlich auf einen erhöhten Energiebedarf, bei gleichzeitig begrenzter Nahrungszufuhr zurückzuführen. Obwohl die Nahrungszufuhr unter erhöhter CO_2-Konzentration limitiert war, waren das Überleben und Wachstum der Heringslarven nicht beeinträchtigt. Dies lässt auf eine Toleranz von Heringslarven gegenüber direkten Effekten der getesteten CO_2-Konzentration schließen. In zwei weiteren Laborexperimenten wurde der direkte Effekt der gleichen CO_2-Konzentration auf Dorschlarven von zwei verschiedenen Populationen, aus der westlichen Ostsee und der Barents See, untersucht. Hierbei zeigte sich, dass eine erhöhte CO_2Konzentration das Überleben von Dorschlarven erheblich reduziert. Legt man diese Daten Berechnungen zur Bestandsentwicklung beider Populationen zugrunde wäre von einer Abnahme auf 8 und 24% der bisherigen Nachwuchsproduktion beim Dorsch auszugehen. In der letzten Studie wurde dann der direkte physiologische, mit dem indirekten nahrungsabhängige Effekt der Ozeanversauerung kombiniert, indem das Überleben und Wachstum von Heringslarven in einem zukünftigen Nahrungsnetz simuliert wurde. Zu diesem Zweck wurde eine natürliche Planktongemeinschaft in Mesokosmen eingeschlossen und einer CO_2 Konzentration ausgesetzt, wie sie für das Ende des 21. Jahrhunderts prognostiziert wird. Die Heringslarven schlüpften in den Mesokosmen und fraßen ausschließlich die mit ihnen eingeschlossenen Nahrungsorganismen. Es stellte sich heraus, dass das Überleben der Heringslarven in Mesokosmen mit erhöhetem CO_2 gesteigert war. Dieser Effekt beruhte auf einer höheren Anzahl von Nahrungsorganismen, welche wiederum auf eine von CO_2 verstärkte Primärproduktion und Phytoplankton-Biomasse zurückzuführen ist. Trotz des positiven, indirekten Effektes von Ozeanversauerung konnte man kein verstärktes Wachstum der Heringslarven feststellen. Dies könnte an der höheren Anzahl von überlebenden Heringslarven in den Mesokosmen mit erhöhter CO_2-Konzentration gelegen haben. Die höhere Individuenzahl hat potenziell einen stärkeren Frassdruck ausgeübt und somit zu einer geringeren Anzahl von Futterorganismen pro Larve geführt. Die größere Konkurrenz zwischen den Heringslarven, durch die gesteigerte Überlebensrate, hat somit vermutlich verhindert, dass auch das Wachstum der Heringslarven von dem positiven, indirekten Ozeanversauerungs-Effekt profitieren konnte. Die Ergebnisse dieser Studien zeigen wie unterschiedlich die direkten Effekte der für das Jahr 2100 prognostizierten CO_2-Konzentration bei toleranten und sensitiven Arten ausfallen können und wie Fischlarven indirekt über die Nahrungskette von Ozeanversauerung beeinflusst werden können.

Folglich ist zu erwarten, dass Fischlarven von CO_2-toleranten Arten wie dem Hering, von Ozeanversauerung zukünftig profitieren, wenn sich zeitgleich das Nahrungsangebot erhöht. Ge-

nauso könnten sie aber auch negativ beeinflusst werden, wenn sich die Nahrungsverfügbarkeit durch Ozeanversauerung verringert. Für die Fischlarven von CO_2-sensitiven Arten wie dem Dorsch, könnte ein reduziertes Nahrungsangebot eine gleichbleibende, wenn nicht sogar verstärkte Beeinträchtigung bedeuten.

Eine interessante Frage, die sich aus den Ergebnissen dieser Dissertation ergibt, ist, ob die negativen Effekte bei CO_2-sensitiven Fischlarven durch ein von Ozeanversauerung verstärktes Nahrungsangebot abgeschwächt oder sogar ausgeglichen werden könnten. Somit sollten zukünftige Studien untersuchen, wann Ozeanversauerung zu einem erhöhten oder verringerten Nahrungsangebot für Fischlarven führt. Außerdem sollte untersucht werden, welchen relativen Einfluss der direkte und indirekte Effekt von Ozeanversauerung auf Fischlarven hat, vor allem wenn mit einem erhöhten Energiebedarf durch die Erwärmung der Ozeane gerechnet werden muss.

Contents

1 | Introduction

1.1 Early life stages of fish in a future ocean

1.1.1 Increasing CO_2 emissions: cause and effects

The anthropogenic burning of fossil fuels and changes in land use increase carbon dioxide (CO_2) levels in the atmosphere and have major impacts on the world's climate and ecosystems (Zeebe, 2012). Over the past thousand years pCO_2 levels in the atmosphere ranged between ~170-300 μatm, but, increased steadily with the onset of industrialization to today's level of ~400 μatm pCO_2(Fig. 1.1) (Lüthi et al., 2008; Le Quéré et al., 2016; Dlugokencky and Tans, 2015). Future emission scenarios suggest an additional dramatic increase of up to 700-1000 μatm pCO_2 by the end of the century (Caldeira and Wickett, 2005). This increase in atmospheric CO_2, one of the most important greenhouse gases, causes the rise in global temperatures, which is also called global warming (Fig. 1.1).

The ocean plays a key role in mitigating global temperatures increases, by absorbing heat from the atmosphere, resulting in ocean warming (OW). During the 20th century, the global oceans average temperature increased by 0.85°C (Stocker et al., 2013). Future scenario models have estimated a further increase by at least 1.5°C by the year 2100 (Stocker et al., 2013). Ocean warming affects life in the ocean, since temperature governs metabolic processes, and most organisms live in a defined temperature range (Pörtner, 2010). Ocean warming has already changed species-specific geographical distributions, e.g. poleward migration, and increased the risk of local extinctions (Perry et al., 2005; Poloczanska et al., 2013; Beaugrand et al., 2002; Overland et al., 2004; Drinkwater, 2006; Parmesan and Yohe, 2003).

Ocean acidification, also dubbed "the other CO_2 problem", relates to the oceans uptake of approximately one third of the anthropogenic CO_2 released into the atmosphere (Doney et al., 2009; Sabine et al., 2004). CO_2 reacts with seawater (H_2O) forming carbonic acid (H_2CO_3), which dissociates into hydrogen (H^+), bicarbonate (HCO_3^-) and carbonate ions (CO_3^{2-}), which leads to a decrease in ocean pH, and an alteration in the carbonate chemistry composition and buffer capacity of the oceans (Fig. 1.2), (Eqn. 1.1) (Doney et al., 2009; Caldeira and Wickett, 2005; Feely et al., 2004).

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons 2\,H^+ + CO_3^{2-} \tag{1.1}$$

The decrease in pH, expressing the increase in hydrogen ion concentration, in the surface of the ocean has been estimated to be ~0.1 pH units from pH 8.21 to 8.10, which resembles an increase in H^+ concentration of 26% since the beginning of industrialization until today. Future projections forecast a further decrease by 0.3-0.4 pH units when pCO_2 levels ~800 μatm are reached by the year 2100 (Stocker et al., 2013). CO_2 levels and, thus, also concomitant effects are expected to differ between regions, latitude, depth, and habitat ((Lee et al., 2003; Sabine et al., 2004; Khatiwala et al., 2013; Orr et al., 2005), (Fig. 1.2).

The impact of OA on marine fauna and ecosystem processes has been of increasing concern not only to scientists but also society, owing to the potential effects on economical and ecosystem services (Fabry et al., 2008; Byrne, 2011; Koenigstein et al., 2016). Changes in pH and the carbonate system can affect ecosystems in the oceans in multiple ways. For example, photosynthesis of primary producers may increase due to higher carbon availability (Rost and Riebesell,

2004; Palacios and Zimmerman, 2007; Zondervan, 2007), whereas calcification may decrease, due to lower calcium carbonate saturation states (Orr et al., 2005; Gattuso et al., 1998; Langdon et al., 2000; Langdon and Atkinson, 2005). Increased photosynthesis could lead to higher phytoplankton biomass and may thus increase the availability of biomass for higher trophic levels. In contrast, lower pH affects the formation of calcium carbonate, which may obviously affect taxa with calcified structures, most prominently coccolithophores, foraminifera, corals, ,echinoderms,mollusks and pteropods (Michaelidis et al., 2005; Hoegh-Guldberg et al., 2007; Siikavuopio et al., 2007; Lischka et al., 2011). Nevertheless, CO_2 tolerant species among these sensitive taxa exist, which maintain calcification or are unaffected by OA, as shown for blue mussels, cuttlefish and corals (Thomsen et al., 2010; Gutowska et al., 2008; Crook et al., 2012).

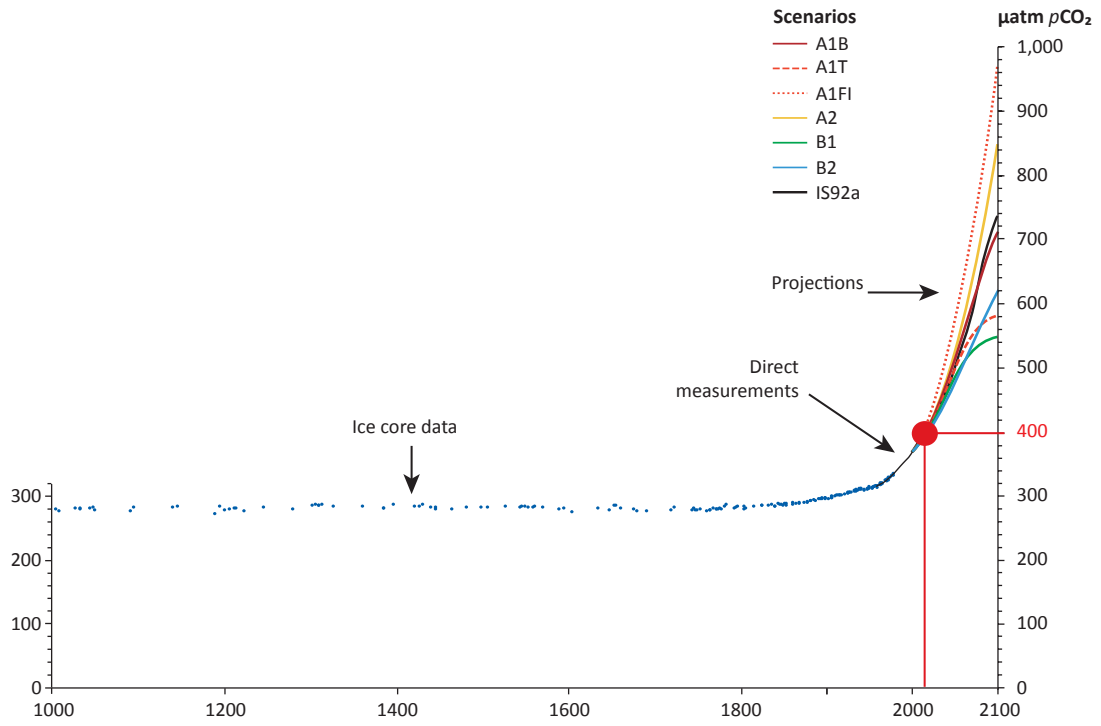

Figure 1.1: Past, present and projected, future atmospheric CO_2 concentrations. Present CO_2 concentrations were measured directly, while past concentrations are estimated from ice cores. Future projections are displayed for the respective emission scenarios (Stocker et al., 2013). Source: http://www.grida.no/publications/vg/climate2, designed by: Philippe Rekacewicz, Emmanuelle Bournay, UNEP/GRID-Arendal.

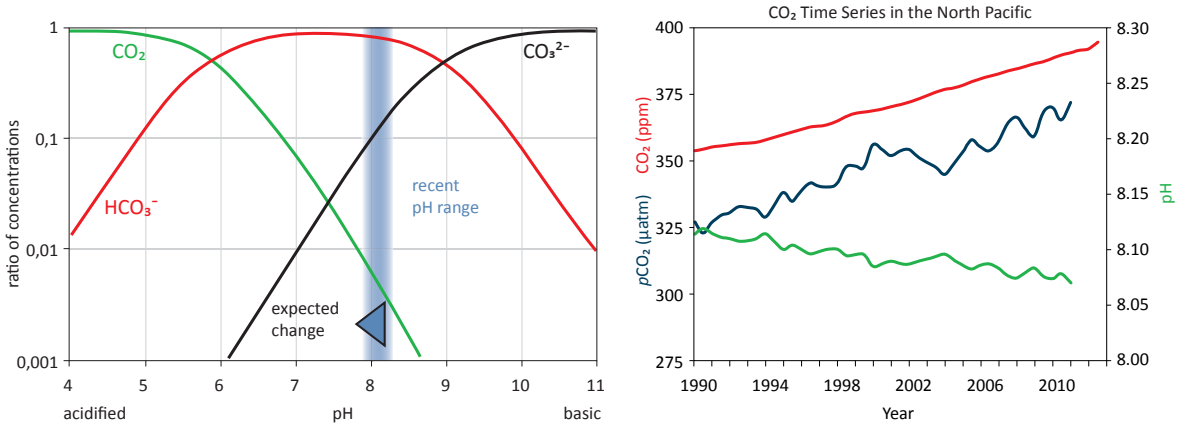

Figure 1.2: **(a)** Relative concentrations of inorganic carbon species (CO_2, HCO_3^-, and CO_3^{2-} dissolved in seawater in relation to pH, at a temperature of 15°C and a salinity of 35 with equilibrium constants calculated after Luecker et al (2000). Present pH range is depicted by the blue area, while the arrow indicates the future direction of change in pH, modified after citepGattuso2011. **(b)** A smoothed time series of atmospheric CO_2 mole fraction (in ppm) at the atmospheric Mauna Loa Observatory (top red line), surface ocean partial pressure of CO_2(pCO_2: middle blue line), and surface ocean pH (bottom green line) at Station ALOHA in the subtropical North Pacific north of Hawaii for the period from1990-2011. The results indicate that the surface ocean pCO_2 trend is generally consistent with the atmospheric increase but is more variable due to large-scale interannual variability of oceanic processes. Modified after IPCC (Stocker et al., 2013).

1.1.2 Ocean acidification and fish

Fish hold important trophic positions in aquatic ecosystem. On the one hand, they are predators on pelagic and benthic organisms such as small crustaceans, while on the other hand, they themselves are preyed upon by higher trophic levels, such as larger carnivorous fish, mammals and birds. Alterations in fish populations e.g. from ocean acidification may, therefore, impact whole ecosystems. Humans rely on fisheries as a major contributor of protein source, with 93.4 million tons of fish caught in 2014, which points to an average consumption of 20.1 kg of fish per capita (FAO, 2016). Thus future changes in fish stocks, caused by climate change, are also of great economical interest.

Although fish are not calcifiers, they may still experience the impacts of OA. Elevated CO_2 levels can lead to acidosis of the blood and surrounding tissues (Brauner and Baker, 2009; Heuer and Grosell, 2014), with potential effects on metabolism, osmoregulation and respiration (Pörtner et al., 2004; Michaelidis et al., 2007; Ishimatsu et al., 2008)(Fig. 1.3). Adult fish and juveniles are considered to be tolerant to CO_2in an OA-relevant range due to their effective acid base regulation in the gill cells (Fig. 1.3)(Melzner et al., 2009; Claiborne et al., 2002; Esbaugh et al., 2012). CO_2 produced by respiration and passively taken in from the environment diffuses, from the blood into the gill cells, where the enzyme carbonic anhydrase catalyzes the reaction of CO_2 with H_2O into H^+ and HCO_3^- ions (review in (Gilmour and Perry, 2009) and references therein). Both ions are then actively transported from the gills into the seawater to regulate pH, in exchange for other ions, e.g. sodium and chloride ion (Gilmour and Perry, 2009). In the kidney bicarbon-

ate is reabsorbed and returned into the blood ((Gilmour and Perry, 2009). Bicarbonate in the blood reacts with hydrogen ions forming CO_2(Gilmour and Perry, 2009; Perry and Gilmour, 2006). This reaction is an important pH buffering mechanism to maintain a certain internal pH, needed for pH-dependent physiological processes, such as enzymatic reactions (Gilmour and Perry, 2009). Hence, although pH homeostasis may be maintained under OA, increased energy demand for these regulating processes may decrease the amount of energy available for other processes, with possible consequences e.g. for reproduction and growth.

Potential effects of OA on the early life stages of fish, eggs and larvae, are highly relevant for population development, since these stages form a critical bottleneck for recruitment (Houde, 2008; Cushing, 1990). In contrast to adult fish, eggs and larvae are considered highly sensitive to OA, due to the lack of effective acid-base regulating organs (Ishimatsu et al., 2004, 2008; Kikkawa et al., 2003). Nevertheless it has been shown that other mechanisms, such as osmoregulation via the skin, chloride cells (ionocytes) and accessory structures exist in early life stages, before subsequently gills and kidneys become the main organs for osmoregulation (Melzner et al., 2009; Ishimatsu et al., 2004; Bodenstein, 2012; Wales and Tytler, 1996). Most studies on eggs and newly hatched larvae found relatively small effects of OA, though species-specific differences were observed (e.g. (Hurst, 2013; Baumann et al., 2011; Franke and Clemmesen, 2011)). For example embryonic duration, egg survival and size at hatch showed no significant impacts at $\sim$1800 μatm pCO_2 in Atlantic herring, *Clupea harengus* (Franke and Clemmesen, 2011). However, embryo survival and size was decreased at $\sim$1000 μatm pCO_2 in Inland silversides, *Menidia beryllina* (Baumann et al., 2011). Stunted growth was also reported for older larvae at elevated CO_2levels, e.g. in Atlantic herring (Frommel et al., 2014), while other species even experienced increases in larval length and weight, e.g. Atlantic cod, *Gadus morhua*, and orange clownfish, *Amphiprion percula* (Munday et al., 2009b; Frommel et al., 2012). The increase in weight was related to altered pathways in protein and lipid biosynthesis (Frommel et al., 2011), while decreased weight under OA was hypothesized to have resulted from changes in organ functionality e.g. in pancreas, liver and kidneys (Frommel et al., 2014).

Fish otoliths, also called "ear stones", are structures used for orientation and hearing. Otoliths consist mostly of calcium carbonate (aragonite) deposited in a protein matrix and thus otolith calcification can be affected by OA. Counter-intuitively otoliths increase in size under OA, as shown for larval Atlantic cod, *Gadus morhua* (Maneja et al., 2013) and cobia, *Rachycentron canadum* (Bignami et al., 2013) at pCO_2 levels > 1800 μatm. The mechanism behind this increase in otolith size relates to an increased bicarbonate concentration at the site of otolith formation, which facilitates calcification (Checkley et al., 2009). Alterations in otolith growth can have the potential to affect larval behavior in terms of hearing and orientation (Bignami 2013). Impacts of elevated CO_2 levels on behavior and sensory functions were also related to the interaction of bicarbonate and the neurotransmitter "GABA" (Nilsson et al., 2012). Under ambient CO_2 concentrations, GABA binding to the receptor leads to a hyperpolarization and thus inhibition of neural activity. At elevated CO_2 levels the ion gradient is reversed, and GABA binding results in depolarization and excitation, which in turn alters behavioral responses (Nilsson et al., 2012; Lopes et al., 2016). A wide range of possible behavioral modifications under OA have been so far discovered in multiple species ranging from predator avoidance, to swimming performance and homing ability (Munday et al., 2009a; Devine et al., 2012; Dixson et al., 2010; Cripps et al., 2011; Simpson et al., 2011; Domenici et al., 2012; Chivers et al., 2014; Maneja et al., 2012,

2015).

OA-induced alterations in growth and behavior can be assumed to result in consequences for larval survival (Nagelkerken and Munday, 2016; Rossi et al., 2016). Also sub-lethal morphological damage, as in the larvae of Atlantic cod, herring and summer flounder (*Paralychtis dentatus* at pCO_2 levels > 1800 μatm (Chambers et al., 2014; Frommel et al., 2011, 2014), has the potential to increase mortality. Nevertheless, survival was rarely measured directly at ocean acidification relevant CO_2 levels. In the few studies published so far, contrasting results have been found: Inland silverside and summer flounder larvae exhibited severely reduced survival from OA (Baumann et al., 2011; Chambers et al., 2013), while European sea bass, *Dicentrachus labrax* and spiny damselfish, *Acanthochromis polyacanthus* were resilient in terms of mortality (Pope et al., 2014; Munday et al., 2011).

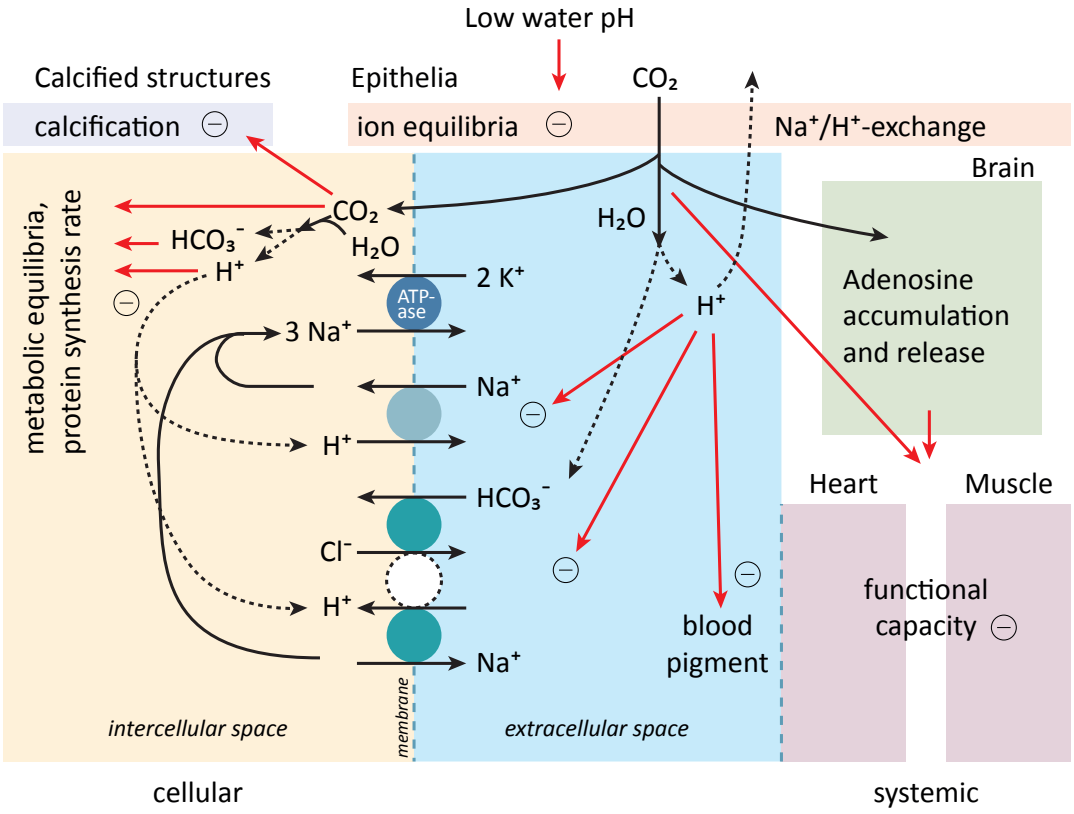

Figure 1.3: Summary of physiological functions and their changes and interactions under the effect of CO_2 in a generalized marine water breathing animal. Note that this picture is incomplete and hypothetical with respect to some details and ignores the specific phylogenetic constraints characterizing individual phyla and species. The generalized cellular processes depicted on the left probably have their specific functional consequences in tissues like brain, heart or muscle depicted on the right. Modified after (Pörtner et al., 2004).

1.1.3 Interaction of OA and OW

As previously explained, ocean acidification and ocean warming occur simultaneously, making an interacting impact in the future ocean highly plausible. To date, few studies have been performed on the interacting effect of elevated temperature and CO_2 levels in early life stages of species of commercial interest. The early life stages of Senegalese sole showed strong negative impacts on hatching success and survival at pCO_2 levels $\sim$1600 μatm and 4°C warmer temperatures than average summer sea surface temperature (Pimentel 2014b). Both, larval growth and metabolism were positively affected by increased temperature but negatively affected by an increased pCO_2 (Pimentel et al., 2014). A synergistic effect of elevated temperature and CO2 was detected as an increase in physical deformities and otolith size. In contrast, larval European seabass displayed higher survival under elevated CO_2 levels (400 vs. 750 μatm pCO_2) and temperatures (17 vs. 19°C) (Pope et al., 2013), leading to, heavier juveniles in the warm treatment and lower aerobic scope under elevated CO_2 levels (Pope et al., 2013). A lower aerobic scope was also reported for adult coral reef fish (Munday et al., 2009a). The aerobic scope in these studies describes the difference between standard and maximum aerobic capacity (Pörtner and Peck, 2010) and can be seen as the extra energy above standard energy demand, which is, for example, available to react to environmental changes (Fry, 1971). Usually studies on the effect of elevated temperature and CO_2 on larval performance are performed at *ad libitum* food densities, meaning excess food. A positive effect of temperature within the optimal range of the species is generally observed for growth, when the energy demand for processes such as metabolism and acid base regulation is met by the food supply (Pörtner, 2010; Oeberst et al., 2009; Folkvord et al., 2004; Feet et al., 2002). Low prey availability and, thus, limited energy uptake, combined with an increased energy demand, may result in less energy being available in total for growth and maintenance when environmental conditions are less than optimum e.g. under OA and OW (Pörtner and Peck, 2010) and references therein). The risk of starvation also increases at elevated temperatures and low food levels, as shown in two age groups of herring larvae (Suneetha et al., 1999), highlighting the need to include energy availability, i.e. temperature and feeding conditions, as interacting factor in OA studies. This points to another important effect that could be caused by future ocean changes and which has been neglected so far in ocean acidification studies on fish. The early life stages of fish may not only be directly affected physiologically but also indirectly via OA-induced changes in the lower food web and thus energy supply.

1.1.4 Indirect effect of OA via the food web

Due to the various effects on calcifying and non-calcifying organisms, whole communities and interactions between trophic levels can be expected to change (Kroeker et al., 2013). This may include the availability of food for higher trophic levels, but also species competing for food sources and their predators. The majority of fish species rely on small zooplankton, such as copepod nauplii, as prey in the larval stage, though intra- and interspecific differences may exist ((Peck et al., 2012; Llopiz, 2013) and references therein). For example, calanoid copepod nauplii and copepodites are the preferred prey of both, Atlantic cod and herring in the first feeding stage (Checkley, 1982; Robert et al., 2013). In recent studies microzooplankton was also considered as a prey component in both species, with herring larvae even ingesting phytoplankton, such as diatoms (Hunt von Herbing and Gallager, 2000; Illing et al., 2015; Denis et al., 2016).

Prey organisms for fish larvae, for example copepods and microzooplankton, are thought to be relatively tolerant to ocean acidification (Nielsen et al., 2010; Burkhardt et al., 1999; Kurihara and Ishimatsu, 2008; McConville et al., 2013), although the effects on several physiological processes, such as metabolism, growth and survival have been reported (Pedersen et al., 2014, 2013; Lewis et al., 2013). When organisms are CO_2-tolerant indirect OA effects, via alterations in the phytoplankton community composition, may become more important (Rose et al., 2009; Calbet et al., 2014). In community based approaches using large mesocosms in the Norwegian Sea, the development of micro- and mesozooplankton was found to be unaffected by elevated CO_2 levels, although a negative impact of OA on copepod nauplii recruitment was indicated (Suffrian et al., 2008; Carotenuto et al., 2007). The effect of OA on microzooplankton in a large scale mesocosm study, in the Baltic Sea, revealed lower diversity at elevated CO_2 levels, with a dominance of smaller ciliates, whereas the total zooplankton abundance, and diversity, was unaffected by OA (Lischka et al., 2015). During another large-scale mesocosm study on the effect of OA on a plankton community from Arctic waters, abundances and community composition of the enclosed micro- and mesozooplankton were unaffected by increased pCO_2 (Aberle et al., 2013; Niehoff et al., 2013), although elevated CO_2 levels altered phytoplankton community composition, with diatoms being negatively impacted, whereas autotrophic dinoflagellates benefitted (Brussaard et al., 2013; Leu et al., 2013; Schulz et al., 2013). In general small-sized phytoplankton, primarily pico-phytoplankton species, are reported to benefit from higher carbon availability under OA, while more variable effects were found for other phytoplankton groups like diatoms, haptophytes and other nano-flagellates, underlining the potential of OA to alter phytoplankton community structure (review in (Riebesell and Tortell, 2011).

Alterations of phytoplankton community composition, and size structure, were shown to affect food quality, e.g. essential fatty acids (EFA), for higher trophic levels (Dalsgaard et al., 2003; Paulsen et al., 2013; St. John, 2001). A cascading OA effect via EFA was shown from primary producers to copepods by (Rossoll et al., 2012), but higher trophic levels like fish larvae have not yet been studied. Fish larvae cannot produce EFA themselves, and thus, fully depend on the transfer of EFA from their prey (Fraser and Sargent, 1989; Navarro et al., 1993; Rossi et al., 2006). Changes in the EFA profiles of prey organisms can have major consequences for fish e.g. influencing fish larval growth (Paulsen et al., 2013; St. John, 2001). Thus, early life stages of fish can be influenced in multiple ways by OA, both directly by interacting with physiological processes, and indirectly via the community in terms of food quantity and quality.

1.1.5 Effect of spatial and temporal overlap

Another important factor influencing fish larval growth, survival and recruitment is spatial and temporal overlap (synchrony) with their respective prey organisms, which forms the basis for the match/mismatch hypothesis formulated by (Cushing, 1990). For example a longer temporal overlap (match) between haddock larvae and the phytoplankton spring bloom, usually followed by increased zooplanktonic prey abundances, positively affected haddock recruitment (Platt et al., 2003). A higher probability of match between offspring, and the onset of the spring bloom, may also be the explanation for Norwegian spring-spawning herring migrating further south despite higher availability of suitable spawning grounds in the north (Vikebø et al., 2012). Hence a mismatch may occur when changes in phenology arise, either in phytoplankton blooms and zooplankton succession, or spawning times and sites of fish species (Cushing, 1990; Fortier

et al., 1995). Ocean warming has already been shown to affect the temporal development of multiple trophic levels in the North Sea, including fish larvae, highlighting differences between functional groups in the response to climate change, and thus, increasing the risk of mismatch between trophic levels (Edwards and Richardson, 2004). For example, a match between cod larvae and high prey abundances resulted in increased cod recruitment in the North Sea between 1960 and 1985, the so-called "gadoid outburst" (Beaugrand et al., 2003). In the following years negative anomalies in the plankton community, most likely induced by increasing temperatures, led to a mismatch, and decreased cod recruitment since the middle of 1980's (Beaugrand et al., 2003). The variability of expected direct, and indirect, OA effects on fish early life stages underline the pressing need for further investigation into the responses of marine fish to ocean acidification, across a wide range of species and under realistic conditions i.e. within natural food webs and temperature regimes (Fig. 1.4).

1.1.6 Life cycle, ecology and fishery of Atlantic herring and cod

Atlantic cod live close to the bottom of the shelf (demersal), usually at 0-200 m water depth (max 900 m), and experience a variety of environmental conditions e.g. in temperature, salinity, oxygen and CO_2 concentrations, owing to their wide distribution in the North Atlantic. Adult cod can live in low salinity waters, such as occurring in the Baltic Sea, but their pelagic eggs rely on a certain salinity to float, either close to the surface such as in the open ocean, or at depth, under the halocline, as in the Baltic Sea (Nissling and Westin, 1997). In the first days after hatch ($\sim$ 5-7 days) the larvae remain in the water column where they hatched and rely on their yolk sac for nutrition (Heath and Lough, 2005). With increasing mobility they start feeding on the small life stages of copepods as preferred prey ((Seljeset et al., 2010; Ottersen et al., 2014) and references therein), subsequently switching to larger prey organisms with increasing size and age. Adult cod are reported to prey on invertebrates, such as mollusks and crustaceans, but also other fish species, as well as cannibalistically on their own kind. In the Baltic Sea a special trophic interaction exists between life stages of cod and the two clupeid species, herring and sprat, *Sprattus sprattus*: Adult cod impart top-down control on populations of both clupeids, whereas adult herring and sprat feed among other planktonic organisms on the cod's eggs and larvae (Rudstam et al., 1994). In the phase of high adult cod abundances, sprat and herring populations are low, and vice versa in phases of increased clupeid dominance (Köster and Möllmann, 2000; Neumann et al., 2014). In addition to natural influences, such as temperature and prey availability, fishing pressure has a major influence on these populations and can trigger switches between the two phases.

Adult Atlantic herring live in swarms in the pelagic realms of the North Atlantic Ocean covering a wide range of spawning seasons but migrating to population-specific spawning locations (review in: (Geffen, 2009). Atlantic herring spawn their "sticky" eggs on hard substrates such as stones and plants on offshore banks or close to the coast (Klinkhardt, 1996). Eggs and larvae experience variable conditions in terms of temperature, light, hydrography and food availability (Heath et al., 1991; Munk, 1992), which herring larvae can react to by adapting their swimming and feeding behavior e.g. by vertical migration (Fortier and Leggett, 1983; Lazzari et al., 1993). Depending on the size of herring larvae, they prey upon a variety of organisms, but different life stages of copepods form the preferred prey (Checkley, 1982; Kiørboe et al., 1985). Herring larvae have been found to survive long periods of little or no growth, enabling survival even in

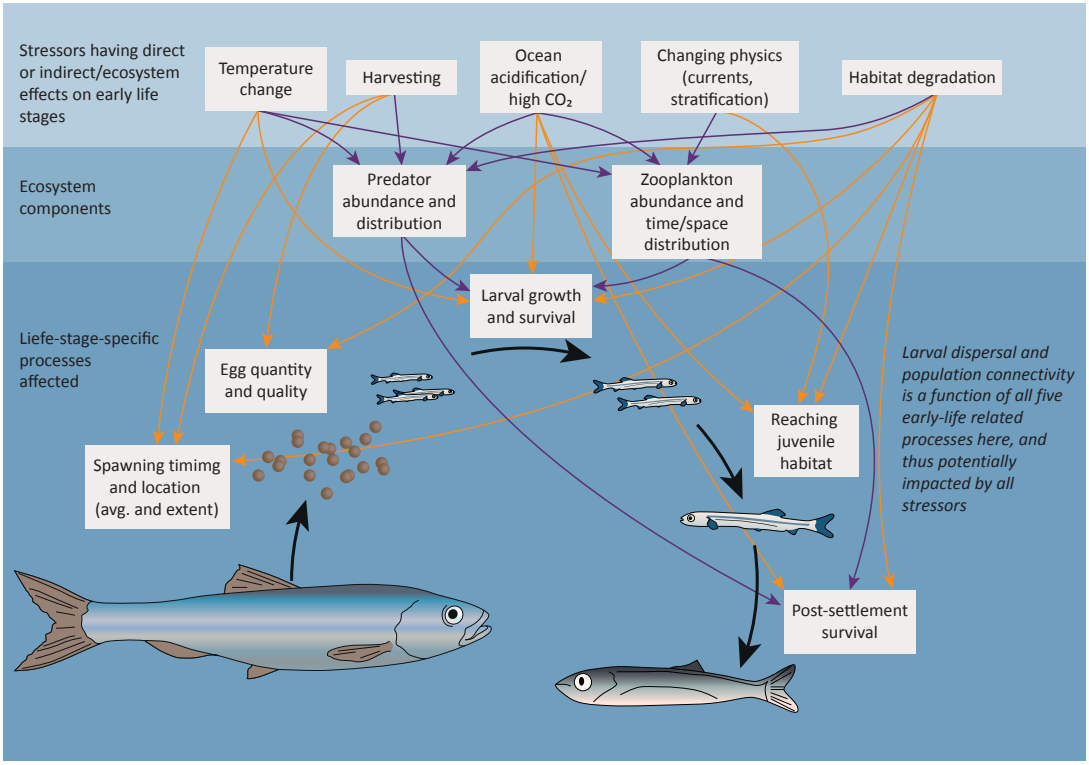

Figure 1.4: Schematic of the potential effects of climate-related and other anthropogenically induced changes on fish early life history. The complexity and potential for interactions of effects are evident from the many arrows from the potential stressors (boxes within light blue area at the top) that are shared by each influential ecosystem component (i.e., predators and prey of early life stages, within blue area second from the top) and early life stage process (deep blue area at the bottom). Dark orange lines are direct effects on early life stages, while purple lines indicate effects on predators and prey of early stages that can, in turn, impact early life survival. Patterns of larval dispersal and population connectivity can vary with variability in all five early life stage processes depicted here, and thus be impacted by all anthropogenic stressors. It is worth noting that this diagram is not exhaustive in the number of potential effects or stressors, and that the stressors are broad (e.g., ?habitat degradation? could range from chemical pollution to sound pollution, pelagic environments to benthic) and are not mutually exclusive (e.g., temperature changes are likely to cause changes in the physics of the ocean, and temperature can be the ultimate or proximate cause of habitat degradation). Further, to reduce complexity, interactions among early life stage process boxes (e.g., egg quality and larval growth and survival) are not included. Modified after (Llopiz et al., 2014).

seasons of low productivity (Johannessen et al., 2000). When larvae are older they are reported to form groups, but the large schools containing billions of fish are not formed before metamorphosis, the developmental transition from the larval to the juvenile stage (Gallego et al., 1995). After metamorphosis, juveniles and adults are able to switch between filter feeding and selective picking of small crustaceans, such as copepods (Batty et al., 1990). Herring itself is an important forage fish for not only other fish, such as cod, but also marine mammals and birds, and is

thus highly important for the Atlantic ecosystem.

Herring and cod are also two of the most important commercially exploited species in the North Atlantic (FAO, 2016). For example in 2014, the German industry processed ∼160.000 t of herring and ∼40.000 t of cod were consumed, corresponding to ∼16% and ∼4% of all fish sold on the market, respectively (Barz and Zimmermann, 2016). Most southern populations of Atlantic cod, e.g. in the North Sea, are considered as overexploited, while the northern populations, e.g. in the Barents Sea, are managed more sustainably (Barz and Zimmermann, 2016). North Atlantic herring populations are considered to be within safe biological limits and managed effectively (Barz and Zimmermann, 2016). Nevertheless the decrease in herring recruitment in the North Sea, observed since 2002, cannot be explained by lower spawning stocks biomass (Payne et al., 2009). Instead increased predation, poor hatching conditions, due to warmer water temperatures and a change in the community composition of the North Sea Plankton, have been identified as possible causes (Alvarez-Fernandez et al., 2015; Petitgas et al., 2013). How ocean acidification may alter community composition, and thus, indirectly affect recruitment in fish populations of commercial interest is so far unknown.

1.2 Thesis Outline

1.2.1 Overview

In light of rising atmospheric CO_2 concentrations, it is crucial to study the potential effects of ocean acidification on the early life stages of fish, eggs and larvae, as these represent a critical bottleneck for recruitment. Fish larvae are considered most susceptible to elevated CO_2 levels, and will be affected by OA in two ways simultaneously, through direct effects on their physiology and indirectly through OA-induced changes in the food web. Earlier studies focusing on the direct impact of OA on fish larvae revealed species-specific and life stage-dependent differences. While detrimental impacts were reported for the larvae of some species, others seem to be tolerant to elevated CO_2 levels. However, the majority of these studies were performed at CO_2 levels higher than expected for the end of the century and may therefor overestimate the possible consequences of OA for the near future. Although it has been addressed in a few studies it remains unclear how the described effects of OA on fish larval performance will affect survival and recruitment of fish populations, especially of commercially important species. The impact of OA may also vary with temperatures and the interacting food effects need to be considered, as most studies to date have been conducted at *ad libitum* (i.e. sufficient) food supply. The dependency between food availability and larval performance is well known, but so far OA effects on fish larval performance, via changes in the plankton community, have not been tested.

Thus the aim of my studies is to address critical questions on how ocean acidification affects the early life stages of two commercially important fish species, Atlantic herring and Atlantic cod, at end of the century CO_2 levels (Fig. 1.5).

This thesis is structured in chapters covering following topics:

Chapter 2 investigates the combined effect of elevated CO_2 levels and temperature on growth and survival of herring larvae. Herring larvae were kept for 32 days in a crossed design of two

temperatures (10°C and 12°C) and two pCO_2 levels (~400 μatm and ~900 μatm) and fed at non-*ad libitum* food conditions with natural plankton from the fjord. Multiple traits such as survival, growth and development were assessed, to examine the effect of elevated CO_2 levels in combination with temperature on herring larval performance.

Chapters 3 and 4 examine the direct physiological and indirect food web effect of ocean acidification on the performance of Atlantic herring larvae. The natural plankton community of the Gullmarsfjord, Sweden was enclosed in large pelagic mesocosms. Five mesocosms served as controls, whereas the remaining five were manipulated to average CO_2 levels of ~760 μatm pCO_2. The plankton community developed for ~9 weeks under the respective CO_2 conditions, before herring larvae hatched inside the mesocosms. After hatch herring larvae lived inside the mesocosm for ~6 weeks and fed exclusively on prey organisms of the enclosed community. Chapter 3 aims to determine the combined direct physiological and indirect food web effect of OA on herring larval survival, whereas Chapter 4 focuses on the direct and indirect effects of OA on growth performance of the survivors.

Chapter 5 presents the direct physiological effect of elevated CO_2 levels on larval survival and recruitment of Atlantic cod from two distinct populations, the Western Baltic and the Arcto-Norwegian Barents Sea stock. Cod larvae from both stocks were kept at ambient and elevated pCO_2 levels (~400-500 μatm and ~1000 μatm, respectively). Larvae from the Western Baltic stock were reared at non-*ad libitum* food densities with natural plankton from the Gullmarsfjord, Sweden for 25 days. The cod larvae from the Barents Sea stock were fed with aquaculture food organisms at low and high food densities with the experiment lasting for 22 days. The resulting effects of elevated CO_2 levels on cod larval survival were incorporated into a Ricker type stock recruitment model for both stocks separately, to test the effect of ocean acidification on recruitment.

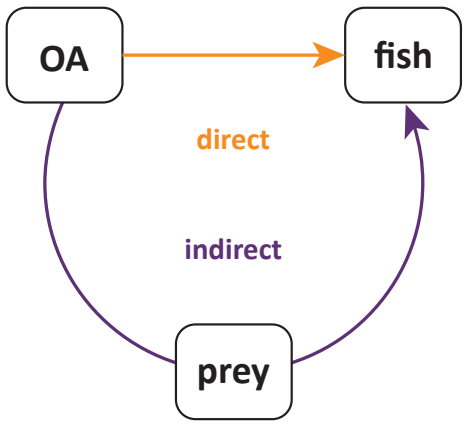

Figure 1.5: Conceptual figure on the direct, physiological and indirect, food web (prey) effects of ocean acidification (OA) on fish larvae (fish).

1.2.2 List of papers for thesis

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

I **Sswat, M.**, Stiasny, M., Jutfelt, F., Riebesell, U. and Clemmesen, C. : Growth performance and survival of larval Atlantic herring, under the combined effects of elevated temperatures and CO_2 *under review*

II **Sswat, M.**, Stiasny, M., Algueró-Muñiz, M., Bach, L. T., Jutfelt, F., Taucher, J., Riebesell, U., Clemmesen, C.: Indirect food web effects of ocean acidification increase herring larval survival, *to be submitted*

III **Sswat, M.**, Stiasny, M., Jutfelt, F., Algueró-Muñiz, M., Horn, H. G., Riebesell, U., Clemmesen, C.: Effect of ocean acidification on prey availability and growth performance of herring larvae in large scale mesocosms. *in preparation*

IV Stiasny, M. H., Mittermayer, F. H., **Sswat, M.**, Voss, R., Jutfelt, F., Chierici, M., Puvanendran, V., Mortensen, A., Reusch, T. B. H. and Clemmesen, C.: Ocean Acidification Effects on Atlantic Cod Larval Survival and Recruitment to the Fished Population, *PLOS One*, 11, 1-11, doi:10.1371/journal. pone.0155448, 2016

1.2.3 Declaration of contribution

Manuscript I:

Idea: Michael Sswat, Catriona Clemmesen, Ulf Riebesell

Data acquisition: Michael Sswat, Catriona Clemmesen, Fredrik Jutfelt, Martina Stiasny

Data interpretation and manuscript preparation: Michael Sswat with comments from all co-authors

Manuscript II:

Idea: Michael Sswat, Catriona Clemmesen, Ulf Riebesell

Data acquisition: Michael Sswat, Maria Algueró-Muñiz, Lennart Bach, Catriona Clemmesen, Fredrik Jutfelt, Ulf Riebesell, Martina Stiasny, Jan Taucher

Data interpretation and manuscript preparation: Michael Sswat with comments from all co-authors

Manuscript III:

Idea: Michael Sswat, Catriona Clemmesen, Ulf Riebesell

Data acquisition: Michael Sswat, Maria Algueró-Muñiz, Lennart Bach, Catriona Clemmesen, Henriette Horn, Fredrik Jutfelt, Martina Stiasny, Jan Taucher

Data interpretation and manuscript preparation: Michael Sswat with comments from all co-authors

Manuscript IV:

Idea: Michael Sswat, Catriona Clemmesen, Martina Stiasny

Data acquisition: Michael Sswat, Catriona Clemmesen, Felix Mittermayer, Martina Stiasny

Data interpretation and manuscript preparation: Martina Stiasny with comments from all co-authors

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2 | Manuscript I

Growth performance and survival of larval Atlantic herring, under the combined effects of elevated temperatures and CO_2

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Abstract

In the coming decades environmental change like warming and acidification will affect life in the ocean. While data on single stressor effects on fish is accumulating rapidly, we still know relatively little about interactive effects of multiple drivers. Of particular concern in this context are the early life stages of fish, for which direct effects of CO_2 on growth and development have been observed. Whether these effects are further modified by elevated temperature was investigated here for the larvae of Atlantic herring, a commercially important fish species. Over a period of 32 days larval survival, growth in size and weight, and instantaneous growth rate were assessed in a crossed experimental design of two temperatures ($10°\mathrm{C}$ and $12°\mathrm{C}$) with two CO_2 levels (400 $\mu\mathrm{atm}$ and 900 $\mu\mathrm{atm}$ $p\mathrm{CO_2}$) at low natural food levels. Elevated temperature alone led to increased activity, as well as decreased survival and instantaneous growth rate $\mathrm{G_i}$. The comparatively high sensitivity to elevated temperature in this study may have been influenced by low food supply. Larval size, $\mathrm{G_i}$ and activity were not affected by CO_2, indicating tolerance of this species to projected "end of the century" CO_2 levels. A synergistic effect of elevated temperature and CO_2 is found for larval weight, with no negative effects detectable at elevated CO_2 concentrations in $12°\mathrm{C}$, but in the $10°\mathrm{C}$ treatment. Contrasting CO_2 effects were found for survival between the two temperatures. Under ambient CO_2 conditions survival was increased at $12°\mathrm{C}$ compared to $10°\mathrm{C}$. In general CO_2 effects are minor and can be considered non-existent compared to the effect of temperature driven by low food supply. These findings emphasize the need to include biotic factors such as energy supply via prey availability in future studies on interactive effects of multiple stressors.

2.1 Introduction

Ocean warming (OW) and acidification (OA), both caused by rising atmospheric CO_2 levels (Le Quéré et al., 2014; Dlugokencky and Tans, 2015), are projected to intensify as an increase in temperature of at least 1.5°C till the end of the century and a decrease in seawater pH of 0.4 units (Howes et al., 2015).These prominent changes will likely interact in their effects on fish populations and are therefore of great interest to society due to the socio-economic value of fishing and other ecosystem services (Haigh et al., 2015; Koenigstein et al., 2016).

The early developmental stages of fish (eggs and larvae) are of particular importance for ecosystems and fisheries since they represent a critical bottleneck for recruitment (Shepherd and Cushing, 1980; Houde, 1987). The performance of fish species and their developmental stages should be best around their abiotic and biotic optimum (Pörtner and Peck, 2010). The theoretical optimal temperature of a species corresponds to optimal (Jobling, 1981) or even suboptimal (Martin and Huey, 2008) growth temperatures and may interact with future CO_2 concentrations as observed for larval European sea bass (Pope et al., 2013) and Senegalese sole (Pimentel et al., 2014b).

The early developmental stages are thought to be most susceptible to changes in pH and therefore most vulnerable to OA (Kikkawa et al., 2004; Brown and Sadler, 1989), as the gills, the main organ for pH regulation are not yet functional during early larval development. Detrimental effects of elevated CO_2 levels, e.g. organ damage (Frommel et al., 2011) and increased embryonic mortality (Forsgren et al., 2013; Stiasny et al., 2016), were reported for the larvae of a range of fish species. Impacts of OA on behaviour (Welch et al., 2014; Pimentel et al., 2014a) are probably caused by an interaction between CO_2 and neurotransmitter function (Nilsson et al., 2012). At the same time no or little effects of OA were found for the larvae of several other species (Munday et al., 2009; Bignami et al., 2014; Hurst, 2013; Schade et al., 2014; Frommel et al., 2012).

Also intraspecific differences of the effect of OA were reported, for example in the early developmental stages and populations of Atlantic herring, *C. harengus*, one of the most important economic fish species in the North Atlantic (Whitehead, 1985). As a coastal bottom-spawning species the eggs and newly hatched larvae of herring can already encounter CO_2 levels higher than expected for the end of the century, due to the seasonal variation and biological activity in coastal areas, especially in the Baltic Sea (Melzner et al., 2009)(von Dewitz, 2012), which could explain these differences in response to OA in performance between populations. No evidence of negative OA impacts, even at 4600 μatm pCO_2, was found for Baltic Sea herring eggs and larvae at hatch on several parameters like hatch rate and size. However nutritional condition in terms of RNA:DNA (Franke and Clemmesen, 2011) and egg survival (Bodenstein, 2012) was found to be negatively affected. In older larvae of Norwegian spring-spawning herring, on the other hand, a negative effect on growth, development, condition and tissue formation was observed at CO_2 levels > 1800 μatm pCO_2 (Frommel et al., 2014), but swimming kinematics and proteome structure were unaffected (Maneja et al., 2014, 2015).

In this study, survival and performance of larval Atlantic herring, *C. harengus*, is investigated under ambient and projected end-of-the-century CO_2-concentrations (400 and 900 μatm

pCO_2) within two temperature regimes (10 and 12°C). We hypothesize that elevated CO_2 levels negatively affect length, weight, instantaneous growth rate, development and survival, while increasing activity (Hypothesis 1), and that these traits are negatively affected in the warmer temperature treatment, due to the low food conditions (Hypothesis 2), leading to a synergistic negative effect of elevated CO_2concentrations and warmer temperatures (Hypothesis 3).

2.2 Materials and methods

4.2.1 Experimental set-up

The experiment was performed at the Sven Lovén Centre for Marine Sciences in Kristineberg, Sweden from April to June 2013. The brood stock herring originated from the Oslo-Fjord close to the Biological Station Drøbak, University of Oslo and were caught using a gillnet on April, 22 nd 2013 at a depth of ∼30m, at the southern tip of Søndre Kaholmen, roughly located at 59°40'29" N and 10°36'22" E. The dead ready-to-spawn herring were transported on ice to the Sven Lovén Centre, where fertilization was performed four hours later. The eggs from five females were stripped separately on 150 plastic plates with an area of 6 cm^2 and ∼150 eggs per plate. Subsequently, the sperm from five males was used to create a total of 25 distinct families, each on six egg plates. Fertilization was performed in seawater of the two CO_2 treatments (∼400 μatm and ∼900 μatm pCO_2), already in line with the treatment the larvae would experience to include possible effects on sperm motility and fertilization success. To synchronize the time of hatching, egg incubation was performed at ambient pCO_2 (400 μatm) conditions and 10°C.

4.2.2 Larval rearing

For the larval rearing two temperatures (10 and 12°C) and two CO_2-levels (ambient: 400 μatm and elevated: 900 μatmpCO_2) were fully crossed and replicated thrice, resulting in twelve tanks in total. The treatments are hereafter named according to their temperature and CO_2 conditions: 10*400, 10*900, 12*400 and 12*900. The two temperature treatments lie within the preferred range (8-12°C) of Atlantic herring larvae described by Mehner et al. (2012). At the time of peak hatch (more than 50% of larvae hatched) the larvae were distributed randomly into plastic bags, which were lowered into the rearing tanks to allow for temperature acclimatization prior to introduction. Larvae were kept at the CO_2 level, at which the eggs had been fertilized, but were randomly distributed between the replicates. The rearing water was taken from the fjord (inlet at 30 m depth), temperature was adjusted before UV treatment and filtration (pore size 500 μm, 50 μm, 20 μm and 5 μm). The treated water was distributed to the rearing tanks of 90 L via twelve individual header tanks of 50 L, where CO_2 manipulation took place via the computer-based pH-System (IKS COMPUTERSYSTEME GMBH). Air bubbling in the header tanks assured mixing and air-water oxygen equilibration. Constant bubbling with tiny pores provided gentle stirring of the rearing tanks, and the daily siphoning of dead prey items and other particles ensured a clean environment for the larvae. Mean water flow-through rate was 200 ml*min per individual header and respective rearing tank, leading to a renewal time of water per rearing tank of ten hours. The light was set according to natural, ambient hours of daylight (16 to 17 hours per day with mean light intensities of 18 μE). Temperature and pH were measured in each tank daily (WTW pH*Cond 340i*3320, WTW Wissenschaftlich-Technische Werkstätten GmbH), while pCO_2 was measured every five days using a method described by Green and Jutfelt (2014) using infra-red CO_2 absorbance (Vaisala, Helsinki, Finland). Temperature and measured pCO_2 over time are shown in Fig. 2.1(a,b). pCO_2 was additionally calculated at the beginning and the end of the experiment using the free software CO_2SYS (Pierrot et al., 2006) with the set constants of (Mehrbach et al., 1973) refitted by Dickson and Millero (1987) and the measured parameters dissolved inorganic carbon (DIC) and total alkalinity (Table 2.1) based on the Best Practices

Guide (Riebesell et al., 2010).

Herring larvae were fed three times per day with natural plankton pumped from the fjord and concentrated with a Hydrotech plankton filter. The prey consisted mainly of calanoid copepod nauplii and copepodites with a size range of 70-200 μm, which are natural prey for herring larvae (Checkley, 1982; Cohen and Lough, 1981; Last, 1980; Sherman and Perkins, 1971). Right after addition mean prey densities of 147 items L^{-1} (minimum 44 and maximum 300 prey items L^{-1} at 8 DPH and 11 DPH, respectively) of rearing water were achieved in each tank (Fig. 2.2) and grazed down till the next feeding event. Mean prey densities are above the critical prey density for herring, estimated as 10 prey items L^{-1} (Rosenthal and Hempel, 1970; Werner and Blaxter, 1980). Laurence (1982) describes a natural range of 5-80 Nauplii L^{-1}, which defines this study to be close to natural prey densities and considerably lower than in other studies with $\sim$2000 prey items L^{-1} (Frommel et al., 2014; Hoie et al., 1999). The low prey densities are also depicted by low sRD values during the study, a proxy for larval nutritional condition, (Fig. 2.3). Additionally a solution of a green algae *Nannochloropsis* sp. ($6*10^5$ cells L^{-1}) was given to create "green water" conditions during the whole experiment, which was shown to have a positive effect on fish larvae (Naas et al., 1992), also recently shown for first feeding herring (Illing et al., 2015).

Sampling and Analysis

Each morning the dead larvae were collected by siphoning the tank floors and counted to calculate mortality rates. Sampling was conducted approximately every five days. After anaesthetization with tricaine methanesulfonate (MS-222) and before freeze storage at minus 80°C a photograph of each individual larva was taken with a digital camera attached to a stereomicroscope (Olympus SZX 7 with Olympus DP 26 Camera and Olympus Stream Essentials Software). Standard length (SL) measurements were gained from calibrated photos of each larva with the open source software Image J (Abràmoff et al., 2004). Developmental stages were determined according to Doyle (1977) and the proportional contribution of each developmental stage was calculated. Dry weight (DW) was measured for each individual larva on a micro balance (Sartorius SC 2 microbalance, Sartorius AG, G"ottingen, Germany, precision $\pm$ 0.1 μg) after freeze drying (Christ Alpha 1-4 freeze dryer, Martin Christ Gefriertrocknungsanlagen GmbH, Osterrode, Germany). The same larvae were later used for nucleic acid analysis according to the protocol described in Malzahn et al. (2003). As an indicator of nutritional condition the ratio of the nucleic acids RNA and DNA (RD) defined as the number of protein biosynthesis machinery per cell was standardized to sRD according Caldarone et al. (2006).

$$\text{sRD} = 0.92 * [\text{RD}] \tag{2.1}$$

Since different temperatures can lead to different activities of these protein biosynthesis units, different estimated growth rates resulting from the total amount of RNA are expected. To compare resulting growth rates from the two temperature treatments, dry weight related instantaneous growth rates (G_i) of the larvae were calculated (Buckley et al., 2008) based on the following relationship:

$$G_i = 0.0145 * [\text{sRD}] + 0.0044 * ([\text{sRD}] * [\text{T}]) - 0.078 \tag{2.2}$$

With G_i being the instantaneous growth rate, sRD the intercalibrated RNA:DNA ratio and

T the temperature determined at the sampling time. A resulting G_i-value of 0 would mean no growth at all and a value of 1 resembles the doubling of the weight of the larva per day. Details on the number of larvae analysed per parameter are given in Supplement (2.4). The activity was measured by automated video tracking software similar to the methods described in detail inSundin and Jutfelt (2015). Briefly, six larvae were placed individually in circular chambers (Ø35 mm, 10 mm water height) and monitored from above using a camera connected to a computer. Six fish from each tank were used. As the larvae were transparent the chambers were placed on a black background and lit by light ramps from the sides, making the larvae appear white. This provided sufficient contrast for reliable tracking of larval movements. The software (Zebralab, Viewpoint, Lyon, France) recorded all movements of each larva (over a certain threshold to avoid image noise) for every frame, and the sampling frequency was 30 frames per second. Each run lasted for 20 minutes. The average one-minute activity for each larva was used for further analysis.

The experiment was divided into two phases based on the nutritional condition (sRD) of the larvae at a time before and after 21 DPH, where a point of inflexion in sRD was observed (Fig. 2.3). Phase I is characterized by a decline, while phase II shows an increasing trend in sRD. To relate larval age in DPH we chose to state degree days (DD) for selected events, which is the cumulative sum of mean daily temperatures for a given age in DPH. According to Fuiman et al. (1998) the best comparison of ontogenetic stages within a species.

The statistical analyses were performed for the whole experimental period and for phase I and phase II separately with the appropriate RStudio packages (Team, 2015). Regression lines of the different parameters for each treatment group and replicate over time in DPH can be found in the supplements (Supplement 2.9). All parameters were analyzed for possible "tank" effects, indicated by significant differences in regression lines of replicates within treatments. A significant effect was detected in the 12*400 treatment, which is the result of a high mortality event in one of the replicate tanks on 21 DPH. The remaining larvae of this tank were sampled until the end of the experiment. In contrast to the two other replicates from 12*400, the "odd tank" experienced a strong increase in length, weight and nutritional condition in the second phase, which indicates an effect of this high mortality event. Since the causes for the higher mortality are unknown and triplicated treatments do not allow for an identification of outliers, this tank is not taken out of the results. The statistical analyses were performed with and without this tank and if differences between treatments occurred, driven by this one tank, it is stated in the results. An analysis of covariance (ANCOVA) approach was chosen to include the two factors temperature and CO_2 as well as the continuous variable time. The ANCOVA-model was fitted to the data on means of standard length, dry weight and G_i per tank and sampling day. For statistical analysis of the activity patterns, each single larva was used in an ANCOVA to include the additional covariate standard length. The assumptions of an ANCOVA, homogeneity of variance, normality and independency of covariates, were assessed visually. The respective likeliness of fit (R^2), degrees of freedom (DF) and the factor's F and p-values are given for each parameter (Table 2.2). The data on developmental stages were calculated as proportions and therefore tested by an ANCOVA design in a generalized linear model with a quasibinomial error distribution. The best model was detected by searching for the smallest Akaike Information Criterion, if differences compared to the more complex models were not significant. For the survival analysis Cox proportional hazards model was chosen (Table 2.3), with exp(coef) being the ratio between mean

survivals and therefore showing which group has a better survival (exp(coef) > 1) in this case means that the described shows a lower survival than the reference group). The survival analysis was repeated for both temperatures separately to discriminate combined effects of temperature and CO_2. The significance level for all statistical analysis was set to $p < 0.05$.

Ethical permit

Animal welfare was assured by performing the experiment according to the ethical permission (number 332-2012) with a separate permit for the behavioural part of the stud (number 151-2011), both issued by the Swedish Board of Agriculture "Jordbruksverket"). In order to minimize stress, specimens were anaesthetized using MS-222 before handling and fixation. The species (*C. harengus*) used is not endangered and was obtained from a local registered and licensed fisherman (licence ID = 977 224 357).

2.3 Results

Effect on larval size

Herring larvae started with a mean standard length (SL) of 9.6 mm $\pm$ 0.5 SD at 2 DPH and reached 13.1 mm $\pm$ 0.8 SD after 32 DPH (Fig. 2.4). The increasing size over time is shown by the significant effect of DPH. Neither temperature nor CO_2 alone showed a statistically significant difference in larval size, but a tendency ($p = 0.11$) towards a combined effect of temperature and CO_2, which becomes significant when the odd tank is excluded in the second phase, was observed (Tables 2.2, Supplement 2.7). This combined effect of temperature and CO_2 in phase II is displayed in higher SL in 10*400 than in 10*900, but lower SL in 12*400 compared to 12*900 (Fig. 2.4).

Effect on larval dry weight

Mean larval dry weight (DW) increased from 0.14 $\pm$ 0.03 mg at 2 DPH to 0.31 $\pm$ 0.09 mg at 32 DPH (Fig. 2.5). A significant effect of CO_2 on weight was detected for phase I, with larger larvae especially in the 10*400 treatment, also detected by the significant interacting effect of temperature and CO_2 on DW over the whole period and during phase I. This combined effect of temperature and CO_2 is depicted in the increased DW in 10*400 compared to 10*900, which could not be found in the 12°C treatments. In phase II this effect is only detectable when the odd tank was excluded (Table 2.2, Supplement 2.7).

Instantaneous growth rate (G_i)

The development of the instantaneous growth rate over time is represented by G_i values of 0.093 $\pm$ 0.028 at 2 DPH, -0.003 $\pm$ 0.022 at 22 DPH and 0.028 $\pm$ 0.027 at 32 DPH (Fig. 2.6). In phase I, when G_i is declining, temperature was found to have a significant effect with in general lower G_i values in 12°C (Table 2.2, Supplement 2.7). From 22 DPH (10°C = ∼225 DD, 12°C = ∼270

DD), when G_i values are lowest, till the end of the study at 32 DPH G_i values are increasing, with temperature having a significant positive effect in this phase II. A significant direct effect of CO_2 was not found. When excluding the odd tank in phase II, temperature and CO_2 together have a significant effect on G_i in phase II, with the pattern matching the development of larval dry weight in phase II.

Survival

Survival displays a steady decline until the end of the experiment (Fig. 2.7). Survival is significantly lower in 12*400 and 12*900 than in 10*400 and 10*900 for the whole period and in each phase I and II alone (Table 2.3). A significant interaction of temperature and CO_2 was found for the whole period and phase I, but not in phase II alone. Higher survival was detected in 10*900 than in 10*400 over the whole period, though there is no significant effects in the single phases I and II. 12*400 shows significantly higher survival than 12*900 in phase I alone, but not for the whole period and phase II (Table 2.3). The difference in survival between CO_2 treatments is in the range of 1-2 days, while $\sim$6 days separate the two temperatures. Also confidence intervals depict the difference between CO_2 treatments in 10°C as small compared to the effect of temperature (Fig. 2.7).

Activity

The activity of the larvae at the end of the experiment (28-30 DPH) was higher in 12*400 and 12*900, and also increased significantly with larval length (Fig. 2.8, Table 2.2, Supplement 2.7). The significant interaction of these two factors can be seen as an increasing temperature effect on the activity of longer larvae. No significant effect of CO_2 on activity was detected.

Developmental stages

The development of the larvae was categorized by determining the Doyle stages. A similar pattern over time with only minor differences between treatment combinations was found with the majority of the larvae reaching stage 2c at the end of the experiment (Fig. 2.9). The statistical analysis showed no effect of temperature or CO_2, neither in the age group 2, 12, 22 or 32 DPH, but detected the ontogenetic development over time (Table 2.2, Supplement 2.7).

2.4 Discussion

Effect of temperature

Although a direct negative effect of warming on Atlantic herring, *Clupea harengus* is thought to be unlikely due to the broad thermal tolerance (Peck et al., 2012b) and both temperatures in the experiment are in the preferred thermal range of herring (Mehner et al., 2012; Reid et al., 1999), a negative effect of higher temperature on survival and instantaneous growth rate was found in this study. Reduced growth rates at higher temperatures in herring have also been reported by

Hoie et al. (1999). In general survival can be matched to the growth potential in this study, with larvae in poor condition at a higher risk for starvation (Clemmesen, 1994). The temperature effects detected in the first phase on instantaneous growth rate and survival may originate from different response times in yolk utilization (Fey, 2001) and the switch from endogenous to exogenous feeding mode (Peck et al., 2012b). After this critical mixed first-feeding stage, the general agreement in the literature states, that increasing growth is related to higher prey densities and temperatures, within the thermal window of the species (Peck et al., 2012b). One possible reason could be increased energy demand at elevated temperatures correlated to increased metabolic rates (Almatar, 1984; Kiørboe et al., 1987), but may also be due to increased activity causing higher active metabolic rates in the warmer temperature. As elevated activity was detected in this study, such a mechanism could be plausible.

The positive effect of temperature on growth regardless of the prey densities, described in an individual based model for herring (Hufnagl and Peck, 2011), is different to our findings, but may be explained by lower prey densities in our study than specified in the model. Usually studies on the effect of temperature on larval growth are performed at ad libitum food densities, so the energy required for processes such as growth and metabolism is met (Pörtner and Peck, 2010) and a positive effect of temperature on growth can be observed (Oeberst et al., 2009; Folkvord et al., 2004; Feet et al., 2002). Energy supply was limited by the low food levels in the current study, and an increased energy demand in the higher temperature may not have been met. An indication for limiting prey densities is given by comparing our data with results on the direct effect of prey abundances on growth in herring larvae (Fox et al., 2003), showing similar larval length at the same age and temperature in their low food treatment. The low food availability is also supported by the low values of $\mathrm{G_i}$, resembling bad feeding conditions (Folkvord et al., 1997; Kiørboe and Munk, 1986; Clemmesen, 1994). Therefore the negative effect of higher temperature is likely a combined direct effect of temperature as well as through increased energy demand. The relatively high $\mathrm{G_i}$ values found in our study right after hatch relate most likely to the endogenous feeding of the larvae on yolk (Peck et al., 2012a), while the slight increase in growth potential to the end of our study cannot be explained by absolute higher food densities, but may result from increasing encounter rates (Johnson et al., 2014) and improvement in hydrodynamic constraints for feeding (China and Holzman, 2014).

Effect of ocean acidification and the interaction with temperature

Herring larval weight was directly negatively impacted at CO_2 levels projected for the end of the century ($\sim$900 $\mu\mathrm{atm}$ $p\mathrm{CO_2}$), though this effect was only significant in the $10^{\circ}\mathrm{C}$ treatment and during the first phase of our study. These findings are in concordance with the literature (Franke and Clemmesen, 2011; Frommel et al., 2014) and may result from an increased energy demand for osmoregulation during the first days after hatch (Kreiss et al., 2015). The fact that larval herring activity (this study) and swimming kinematics (Maneja et al., 2015) were unaffected by CO_2 fits with recent findings that many temperate species appear behaviourally robust to increased CO_2-concentrations (Maneja et al., 2012, 2015; Jutfelt and Hedgärde, 2013, 2015; Sundin and Jutfelt, 2015; Näslund et al., 2015), despite the negative effects on behaviour that have been reported in several tropical fish e.g. by Munday et al. (2013).

Different effects of elevated CO_2 concentrations in the two temperature treatments were also

found for larval weight in phase II, when the "odd" tank was excluded. In 12°C elevated CO_2 had a positive effect on weight, while in 10°C this effect remained negative. It is difficult to hypothesize about responsible mechanisms, especially since this parameter shows no difference between treatments on the last day of sampling. However, both CO_2 effects were described before: Frommel et al. (2014) found a negative effect on growth in weight and suspected altered organ functionality e.g. in pancreas, liver and kidneys as underlying mechanism, while a positive effect was assumed to indicate altered pathways in protein and lipid biosynthesis (Frommel et al., 2011). Both of these mechanisms may affect larval growth efficiency and survival. The interaction of low energy supply needs to be taken into account, which calls for a more thorough investigation, how effects of different temperatures and elevated CO_2 concentrations interact at different food levels.

Interspecific differences might explain the effect of elevated CO_2 concentrations on survival. Distinct negative effects of OA on survival were found for the important forage fish Atlantic silverside (Baumann et al., 2011) and two populations of the economically important Atlantic cod (Stiasny et al., 2016), while in our study survival of Atlantic herring larvae showed contrasting effects depending on the temperature. A negative CO_2 effect on survival was detected in 12°C, but a positive one in 10°C, which calls for a careful interpretation of the data and further research on this topic. Differences in survival between CO_2 treatments range between 1-2 days, which is close to the detection limit of survival rates in our study, since dead larvae were sampled on a daily basis. In general the effect of CO_2 is small compared to the effect of temperature in our study, as can be seen in the small effect sizes of the CO_2 treatments.

Ocean warming is claimed to result in an increased total energy demand for metabolism, while ocean acidification is hypothesized to only increase energy demand for osmoregulation (Kreiss et al., 2015). Limited energy uptake due to low prey availability, combined with an increased energy demand, may result in less energy available in total for growth and maintenance. Additionally the risk of starvation may increase at elevated temperatures, thereby lowering survival, as shown for two age-groups of herring larvae (Suneetha et al., 1999). Probably owing to the increased risk of starvation, one of the replicates in the warmer treatment of our study experienced a high mortality at 21 DPH and afterwards showed a strong increase in growth. Two possible explanations can be imagined for this cause, an increase in food items per individual larvae as well as a selection for larvae with a lower starvation potential. A selection for phenotypes with better growth at unfavourable prey availability was shown already in the field for larval Atlantic cod *Gadus morhua* (Meekan and Fortier, 1996). An increased energy demand, e.g. at elevated temperature or CO_2 conditions, may thus have the potential to select for specific phenotypes and could especially interact with altered energy supply in terms of food availability.

Conclusion

Although there is an indication for a negative CO_2 effect on weight in our study, the overall impression is that herring larvae are tolerant to end of the century CO_2 conditions (Hypothesis 1). Especially when compared to the negative impact of elevated temperatures due to low food supply (Hypothesis 2), with impacts on instantaneous growth rate, size and survival. A synergistic effect of elevated temperatures and CO_2 concentrations (Hypothesis 3) is indicated for herring larval weight and survival, but calls for more detailed investigations, with a focus on

selection processes. The comparatively small effect of end of the century CO_2 concentrations on herring larval performance might be explained by the abiotic conditions already experienced through their life cycle as a bottom spawning species. The diverse results on possible effects of ocean acidification (OA) on the performance of this economically important species, reported in the literature, indicate differences in reaction patterns depending on applied OA scenarios, population and life stages. It also supports the proposition that Atlantic herring may be tolerant to future OA scenarios. For a full understanding of the potential impacts of OA on fish larvae, indirect effects via food web interactions will need to be considered and may have the potential to additionally affect performance of early life stages in fish.

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Tables and figures

Table 2.1: Abiotic factors per treatment combination. T=temperature, pH=-log[H^+], TA=total alkalinity, DIC=dissolved inorganic carbon, CO_2 m=measured pCO_2, CO_2 c=calculated pCO_2(means and standard deviations derived from the various measurements over the experimental time).

Treatment	T	pH	TA	DIC	pCO_2 m	pCO_2 c
	(°C)	(units)	(μmol*kg^{-1})	(μmol*kg^{-1})	(μatm)	(μatm)
10*400	10.4 ± 0.2	8.11 ± 0.05	2320 ± 19	2156 ± 6	475 ± 20	450 ± 58
10*900	10.5 ± 0.3	7.81 ± 0.10	2304 ± 4	2257 ± 12	877 ± 68	982 ± 23
12*400	12.4 ± 0.7	8.10 ± 0.06	2328 ± 11	2128 ± 51	479 ± 15	463 ± 59
12*900	12.7 ± 0.6	7.79 ± 0.08	2328 ± 10	2259 ± 6	938 ± 44	907 ± 61

Table 2.2: Results from ANCOVA models, only significant factors are shown for the different parameters with the respective likeliness of fit (R^2), F-values and p-values. The factors listed are additive for the described models. The complete table can be found in the Supplement.

Parameter	Period	Temperature	R^2	Factor	DF	F-value	p-value
Length	Whole period	10°C and 12°C	0.75	DPH	4, 79	244.78	<0.001
	Phase I	10°C and 12°C	0.75	DPH	4, 43	142.86	<0.001
	Phase 2 - T9	10°C and 12°C	0.34	DPH	5, 27	5.47	<0.05
				T*CO_2		7.49	<0.05
Weight	Whole period	10°C and 12°C	0.70	DPH	4, 79	185.78	<0.001
				T*CO_2		7.31	<0.05
	Whole period	10°C	0.73	DPH	2, 39	106.09	<0.001
				CO_2		7.83	<0.01
	Whole period	12°C	0.66	DPH	2, 39	79.71	<0.001
	Phase I	10°C and 12°C	0.61	DPH	5, 42	63.84	<0.001
				CO_2		5.19	<0.05
				T*CO_2		6.71	<0.05
	Phase I	10°C	0.64	DPH	2, 21	34.95	<0.001
				CO_2		7.40	<0.05
	Phase I	12°C	0.51	DPH	1, 22	28.77	<0.001
	Phase II - T9	10°C and 12°C	0.42	DPH	4, 28	14.07	<0.001
				T*CO_2		11.05	<0.01
Gi	Whole period	10°C and 12°C	0.52	DPH	1, 82	89.25	<0.001
	Phase I	10°C and 12°C	0.89	DPH	4, 43	372.79	<0.001
				DPH*T		18.91	<0.001
	Phase II	10°C and 12°C	0.56	DPH	3, 32	38.64	<0.001
				T		5.39	<0.05
	Phase II - T9	10°C and 12°C	0.68	DPH	5, 27	59.75	<0.001
				T*CO_2		8.09	<0.01
Activity	Final	10°C and 12°C	0.36	SL	6, 56	25.9	<0.001
				T		7.12	<0.01
				SL*T		5.42	<0.05
Development	Whole period	10°C and 12°C		stage		9.3	<0.01
				DPH*stage		55.53	<0.001

Table 2.3: Results of the Cox-PH models for survival with the respective Chi squares, degrees of freedom (DF), p-values and approximate hazards (exp(coef). Treatment values of 12 x 900 refer to °C x μatm pCO_2 and the given interaction.

Parameter	Temperature	Period	Chi	DF	Treatment	p-value	exp(coef)
Survival	10°C and 12°C	Whole period	488.6	3	**12**	**<0.001**	**1.54**
					900	**<0.05**	**0.92**
					12 * 900	**<0.01**	**1.13**
		Phase I	36.29	3	**12**	**<0.05**	**1.09**
					900	0.18	0.94
					12 * 900	**<0.05**	**1.13**
		Phase II	17.84	1	**12**	**<0.001**	**1.21**
	10°C	Whole period	6.2	1	**900**	**<0.05**	**0.92**
		Phase I	1.81	1	900	0.18	0.94
		Phase II	0.69	1	900	0.41	0.95
	12°C	Whole period	1.8	1	900	0.20	1.04
		Phase I	3.95	1	**900**	**<0.05**	**1.06**
		Phase II	0.69	1	900	0.41	0.94

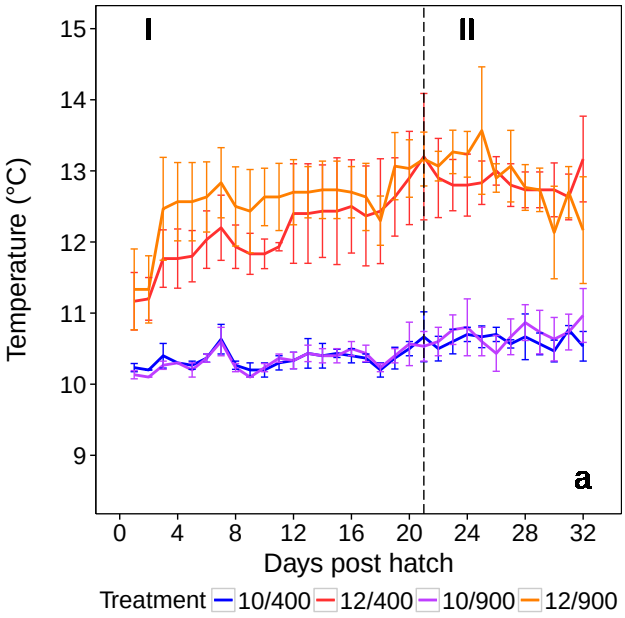

Figure 2.1: Temperature (a) and measured pCO_2(b) per treatment combination (mean and standard error) over the experimental period against days post hatch.

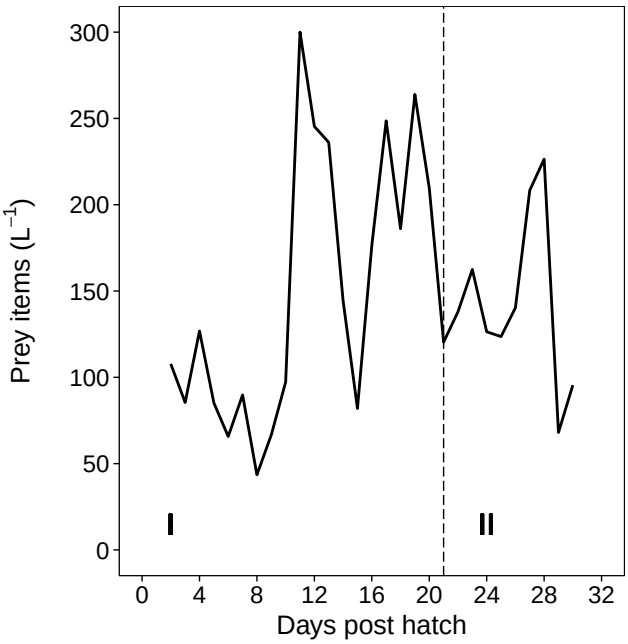

Figure 2.2: Calculated daily prey densities after addition to the rearing tank (in the size range from 70 - 200 μm consisting mainly of calanoid copepods) offered to herring larvae over the experimental period in days post hatch.

Table 2.4: S1 Table: Number of larvae per replicate (Tank) and sampling day in days post hatch (DPH) used for the respective analysis (SL=standard length, DW=dry weight, G_i=growth potential, Doyle=developmental stage, Activity was only measured once at the end).

DPH	Replicate	Treatment	n SL	n DW	n G_i	n Stage	n Activity
2	1	12*400	10	10	10	12	
	2	10*900	10	10	10	10	
	3	10*400	10	10	10	10	
	4	12*900	10	10	10	10	
	5	12*400	10	10	10	10	
	6	10*400	10	10	10	10	
	7	12*900	10	10	10	10	
	8	10*900	10	10	10	10	
	9	12*400	10	10	10	10	
	10	10*900	10	10	10	10	
	11	10*400	10	10	10	10	
	12	12*900	10	10	10	10	
7	1	12*400	10	10	10	10	
	2	10*900	10	10	10	10	
	3	10*400	10	10	10	10	
	4	12*900	10	10	10	10	
	5	12*400	10	10	10	10	
	6	10*400	10	10	10	10	
	7	12*900	10	10	10	10	
	8	10*900	10	10	10	10	
	9	12*400	10	10	10	10	
	10	10*900	10	10	10	10	
	11	10*400	10	10	10	10	
	12	12*900	10	10	10	10	
12	1	12*400	10	10	10	10	
	2	10*900	10	10	10	10	
	3	10*400	10	10	10	10	
	4	12*900	10	10	10	10	
	5	12*400	10	10	10	10	
	6	10*400	10	10	10	10	

Table 2.5: S1 Table, continued: Number of larvae per replicate (Tank) and sampling day in days post hatch (DPH) used for the respective analysis (SL=standard length, DW=dry weight, G_i=growth potential, Doyle=developmental stage, Activity was only measured once at the end).

DPH	Replicate	Treatment	n SL	n DW	n G_i	n Stage	n Activity
12	7	12*900	10	10	10	10	
	8	10*900	10	10	10	10	
	9	12*400	10	10	10	10	
	10	10*900	10	10	10	10	
	11	10*400	10	10	10	10	
	12	12*900	10	10	10	10	
17	1	12*400	10	10	10	10	
	2	10*900	10	10	10	10	
	3	10*400	10	10	10	10	
	4	12*900	10	10	10	10	
	5	12*400	10	10	10	10	
	6	10*400	10	10	10	10	
	7	12*900	10	10	10	10	
	8	10*900	10	10	10	10	
	9	12*400	10	10	10	10	
	10	10*900	10	10	10	10	
	11	10*400	10	10	10	10	
	12	12*900	10	10	10	10	
22	1	12*400	10	10	10	10	
	2	10*900	10	10	10	10	
	3	10*400	10	10	10	10	
	4	12*900	10	10	10	10	
	5	12*400	10	10	10	10	
	6	10*400	10	10	10	10	
	7	12*900	10	10	10	10	
	8	10*900	10	10	10	10	
	9	12*400	10	10	10	10	
	10	10*900	10	10	10	10	
	11	10*400	10	10	10	10	
	12	12*900	10	10	10	10	

Table 2.6: S1 Table, continued: Number of larvae per replicate (Tank) and sampling day in days post hatch (DPH) used for the respective analysis (SL=standard length, DW=dry weight, G_i=growth potential, Doyle=developmental stage, Activity was only measured once at the end).

DPH	Replicate	Treatment	n SL	n DW	n G_i	n Stage	n Activity
27	1	12*400	10	10	10	10	
	2	10*900	10	10	10	10	
	3	10*400	10	10	10	10	
	4	12*900	10	10	10	10	
	5	12*400	9	10	10	5	
	6	10*400	10	10	10	9	
	7	12*900	10	10	10	10	
	8	10*900	10	10	10	8	
	9	12*400	10	10	10	10	
	10	10*900	10	10	10	7	
	11	10*400	10	10	10	9	
	12	12*900	10	10	10	10	
32	1	12*400	10	10	10	7	6
	2	10*900	10	10	10	8	6
	3	10*400	10	10	10	7	6
	4	12*900	10	10	10	9	6
	5	12*400	10	10	10	6	5
	6	10*400	10	10	10	9	6
	7	12*900	10	10	10	10	6
	8	10*900	10	10	10	8	6
	9	12*400	10	10	10	10	5
	10	10*900	10	10	10	9	6
	11	10*400	10	10	10	5	6
	12	12*900	10	10	10	10	6

Table 2.7: S2 Table: Outcome of the best fitting ANCOVA models for the different parameters with the respective likeliness of fit (R^2), F-values and p-values. The factors listed are additive for the described models, e.g. lm: Length∼DPH+T+CO_2+T*CO_2.

Parameter	Period	Temperature	R2	Factor	DF	F-value	p-value
Length	Whole period	10°C and 12°C	0.75	**DPH**	4, 79	**244.78**	**<0.001**
				T		0.11	0.75
				CO_2		0.61	0.44
				T* CO_2		2.56	0.11
	Phase I	10°C and 12°C	0.75	**DPH**	4, 43	**142.86**	**<0.001**
				T		0.02	0.89
				CO_2		2.48	0.12
				T*CO_2		2.46	0.12
	Phase II	10°C and 12°C	0.12				
	Phase II - T9	10°C and 12°C	0.34	**DPH**	5, 27	**5.47**	**<0.05**
				T		**3.60**	**0.07**
				CO_2		2.84	0.1
				DPH*T		2.39	0.13
				T*CO_2		**7.49**	**<0.05**
Weight	Whole period	10°C and 12°C	0.70	**DPH**	4, 79	**185.78**	**<0.001**
				T		0.21	0.65
				CO_2		1.46	0.23
				T*CO_2		**7.31**	**<0.05**
	Whole period	10°C	0.73	**DPH**	2, 39	**106.09**	**<0.001**
				CO_2		**7.83**	**<0.01**
	Whole period	12°C	0.66	**DPH**	2, 39	**79.71**	**<0.001**
				CO_2		1.08	0.31
	Phase I	10°C and 12°C	0.61	**DPH**	5, 42	**63.84**	**<0.001**
				T		0.18	0.67
				CO_2		**5.19**	**<0.05**
				DPH*T		3.97	0.05
				T*CO_2		**6.71**	**<0.05**
	Phase I	10°C	0.64	**DPH**	2, 21	**34.95**	**<0.001**
				CO_2		**7.40**	**<0.05**
	Phase I	12°C	0.51	**DPH**	1, 22	**28.77**	**<0.001**

Table 2.8: S2 Table, continued: Outcome of the best fitting ANCOVA models for the different parameters with the respective likeliness of fit (R^2), F-values and p-values. The factors listed are additive for the described models, e.g. lm: Length∼DPH+T+CO_2+T*CO_2.

Parameter	Period	Temperature	R2	Factor	DF	F-value	p-value
Weight	Phase II	Phase II	0.22				
	Phase II - T9	Phase II - T9	0.42	**DPH**	4, 28	**14.07**	**<0.001**
				T		0.38	0.54
				CO_2		2.12	0.16
				T*CO_2		11.05	**<0.01**
Gi	Whole period	10°C and 12°C	0.52	**DPH**	1, 82	89.25	**<0.001**
	Phase I	10°C and 12°C	0.89	**DPH**	4, 43	372.79	**<0.001**
				T		0.12	0.74
				CO_2		3.32	0.08
				DPH*T		**18.91**	**<0.001**
	Phase II	10°C and 12°C	0.56	**DPH**	3, 32	**38.64**	**<0.001**
				T		**5.39**	**<0.05**
				DPH*T		2.68	0.11
	Phase II - T9	10°C and 12°C	0.68	**DPH**	5, 27	**59.75**	**<0.001**
				T		3.53	0.07
				CO_2		0.39	0.54
				DPH*CO_2		1.98	0.17
				T*CO_2		**8.09**	**<0.01**
Activity	Final	10°C and 12°C	0.36	**SL**	6, 56	**25.90**	**<0.001**
				T		**7.12**	**<0.01**
				CO_2		0.36	0.55
				T*CO_2		1.67	0.2
				SL*CO_2		2.70	0.11
				SL*T		**5.42**	**<0.05**
Development	Whole period	10°C and 12°C		DPH		0	1
				stage		**9.3**	**<0.01**
				DPH*stage		**55.53**	**<0.001**

Table 2.9: S3 Table: Regression lines of the different parameters over time in days post hatch (DPH) for each treatment group and replicate.

Parameter	Treatment	Tank	Formula
Total length	10*400	3, 6, 11	TL= 10.222 + 0.111*DPH
		3	TL= 10.313 + 0.108*DPH
		6	TL = 10.614 + 0.099*DPH
		11	TL = 9.739 + 0.126*DPH
	10*900	2, 8, 10	TL = 9.642 + 0.124*DPH
		2	TL = 9.436 + 0.136*DPH
		8	TL = 9.517 + 0.137*DPH
		10	TL = 9.963 + 0.100*DPH
	12*400	1, 5, 9	TL = 10.096 + 0.101*DPH
		1	TL = 10.232 + 0.076*DPH
		5	TL = 10.731 + 0.057*DPH
		9	TL = 9.350 + 0.168*DPH
	12*900	4, 7, 12	TL = 10.094 + 0.108*DPH
		4	TL = 9.931 + 0.108*DPH
		7	TL = 10.565 + 0.089*DPH
		12	TL = 9.794 + 0.127*DPH
Dry weight	10*400	3, 6, 11	DW = 0.143 + 0.006*DPH
		3	DW = 0.156 + 0.024*DPH
		6	DW = 0.149 + 0.007*DPH
		11	DW = 0.125 + 0.007*DPH
	10*900	2, 8, 10	DW = 0.100 + 0.007*DPH
		2	DW = 0.084 + 0.007*DPH
		8	DW = 0.099 + 0.008*DPH
		10	DW = 0.117 + 0.006*DPH
	12*400	1, 5, 9	DW = 0.118 + 0.006*DPH
		1	DW = 0.133 + 0.004*DPH
		5	DW = 0.165 + 0.002*DPH
		9	DW = 0.058 + 0.013*DPH
	12*900	4, 7, 12	DW = 0.124 + 0.007*DPH
		4	DW = 0.135 + 0.005*DPH
		7	DW = 0.148 + 0.006*DPH
		12	DW = 0.092 + 0.009*DPH

Table 2.10: S3 Table, continued: Regression lines of the different parameters over time in days post hatch (DPH) for each treatment group and replicate.

Parameter	Treatment	Tank	Formula
G_i	10*400	3, 6, 11	G_i= 0.0813 - 0.0024*DPH
		3	G_i= 0.0879 - 0.0031*DPH
		6	G_i= 0.0848 - 0.0021*DPH
		11	G_i= 0.0718 - 0.0022*DPH
	10*900	2, 8, 10	G_i= 0.0741 - 0.0024*DPH
		2	G_i= 0.073 - 0.0023*DPH
		8	G_i= 0.0719 - 0.0023*DPH
		10	G_i= 0.0770 - 0.0025*DPH
	12*400	1, 5, 9	G_i= 0.0800 - 0.0023*DPH
		1	G_i= 0.0864 - 0.0029*DPH
		5	G_i= 0.0858 - 0.0029*DPH
		9	G_i= 0.0682 - 0.0011*DPH
	12*900	4, 7, 12	G_i= 0.0769 - 0.0022*DPH
		4	G_i= 0.0793 - 0.0025*DPH
		7	G_i= 0.0826 - 0.0024*DPH
		12	G_i= 0.0690 - 0.0017*DPH
G_i	10*400	3, 6, 11	G_i= 0.0967 - 0.0040*DPH
		3	G_i= 0.1107 - 0.0057*DPH
		6	G_i= 0.0995 - 0.0034*DPH
		11	G_i= 0.0799 - 0.0029*DPH
	10*900	2, 8, 10	G_i= 0.0906 -0.0040*DPH
		2	G_i= 0.0809 - 0.0029*DPH
		8	G_i= 0.0897 - 0.0041*DPH
		10	G_i= 0.1011 - 0.0051*DPH
	12*400	1, 5, 9	G_i= 0.1121 - 0.0058*DPH
		1	G_i= 0.1107 - 0.0057*DPH
		5	G_i= 0.1152 - 0.0062*DPH
		9	G_i= 0.1105 - 0.0057*DPH
	12*900	4, 7, 12	G_i= 0.1174 - 0.0068*DPH
		4	G_i= 0.1149 - 0.0065*DPH
		7	G_i= 0.1226 - 0.0069*DPH
		12	G_i= 0.1148 - 0.0071*DPH

Table 2.11: S3 Table, continued: Regression lines of the different parameters over time in days post hatch (DPH) for each treatment group and replicate.

Parameter	Treatment	Tank	Formula
G_i	10*400	3, 6, 11	G_i= -0.0473 + 0.0021*DPH
		3	G_i= -0.0332 + 0.0014*DPH
		6	G_i= -0.0746 + 0.0035*DPH
		11	G_i= -0.0253 + 0.0012*DPH
	10*900	2, 8, 10	G_i= -0.0637 + 0.0025*DPH
		2	G_i= -0.0416 + 0.0018*DPH
		8	G_i= -0.0704 + 0.0027*DPH
		10	G_i= -0.0800 + 0.0031*DPH
	12*400	1, 5, 9	G_i= -0.0964 + 0.0042*DPH
		1	G_i= -0.0293 + 0.0014*DPH
		5	G_i= -0.0737 + 0.0029*DPH
		9	G_i= -0.1797 + 0.0080*DPH
	12*900	4, 7, 12	G_i= -0.0865 + 0.0038*DPH
		4	G_i= -0.0652 + 0.0030*DPH
		7	G_i= -0.1144 + 0.0046*DPH
		12	G_i= -0.0771 + 0.0037*DPH

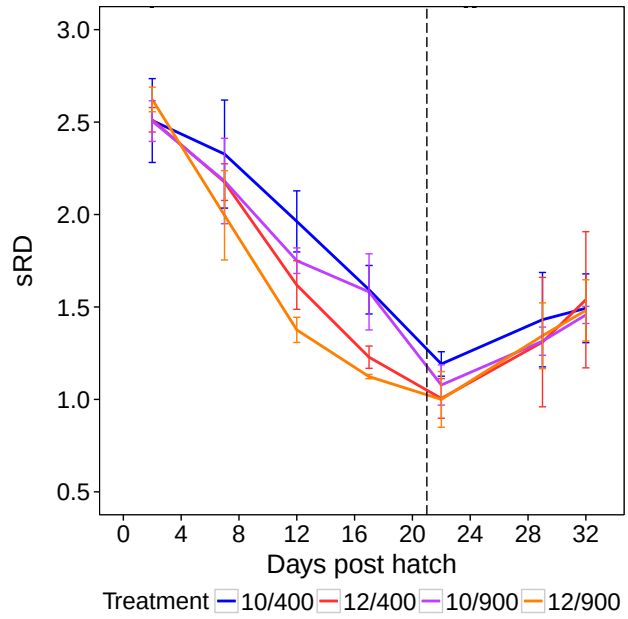

Figure 2.3: Development of nutritional condition (sRD) over time per treatment combination (mean and standard deviation) for the whole experimental period against days post hatch. Vertical line represents the separation into phases I and II.

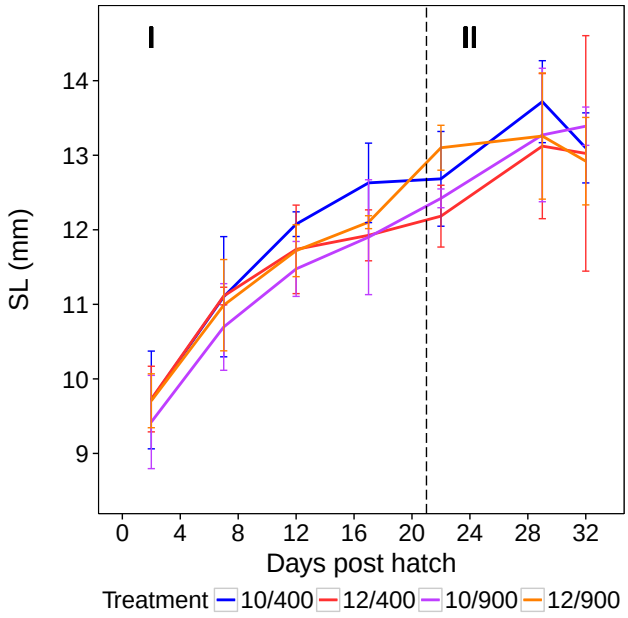

Figure 2.4: Larval standard length (SL) in mm per treatment combination (mean and standard deviation) for the whole experimental period against days post hatch. Vertical line represents the separation into phases I and II.

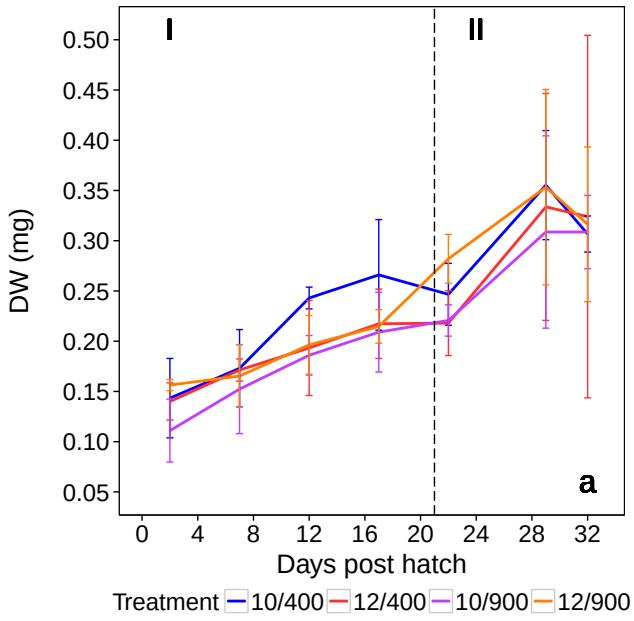

Figure 2.5: Larval dry weight in mg per treatment combination (mean and standard deviation) for the whole experimental period against days post hatch.

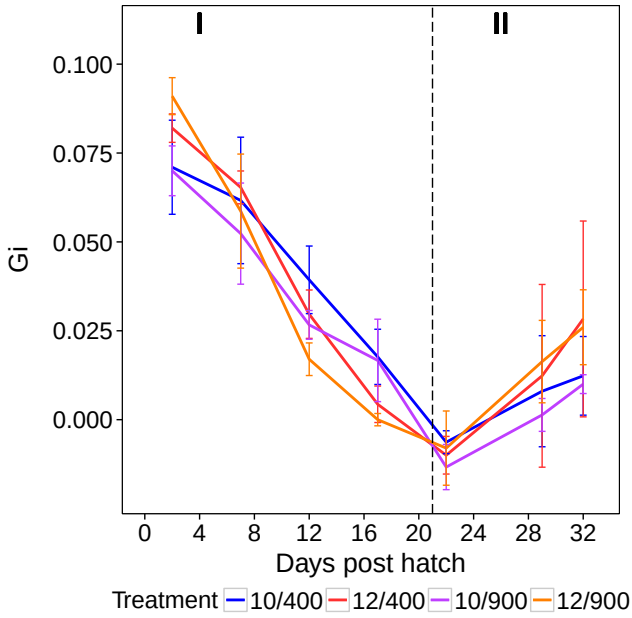

Figure 2.6: Instantaneous growth rate (G_i) per treatment combination (mean and standard deviation) during the experimental period against days post hatch.

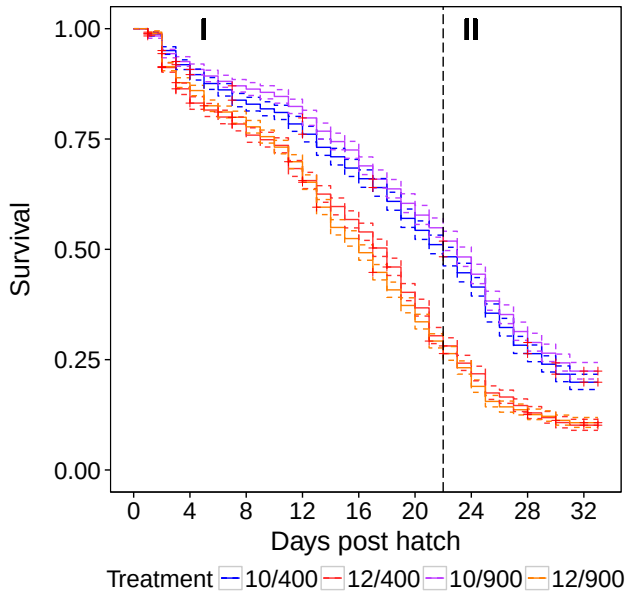

Figure 2.7: Mean larval survival per treatment combination for the whole experimental period against days post hatch.

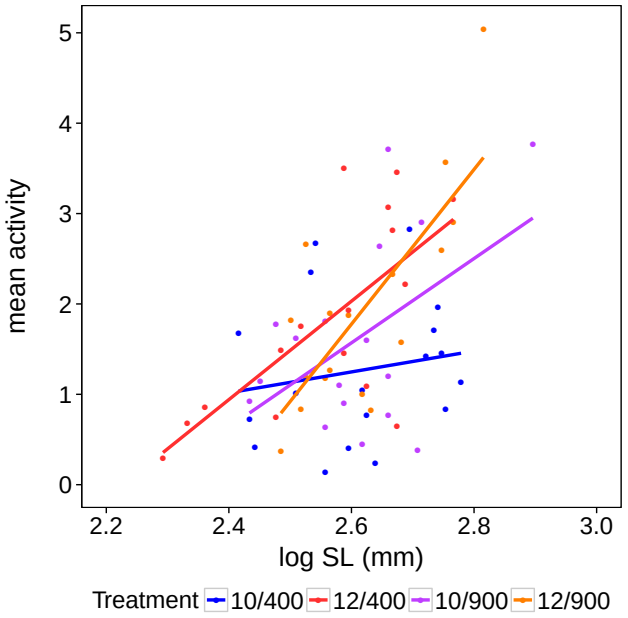

Figure 2.8: Activity plotted over log standard length (SL) (mm) of herring larvae reared in one of the four treatments (10 x 400, 10 x 900, 12 x 400 and 12 x 900) for 32 days prior to the activity measurements.

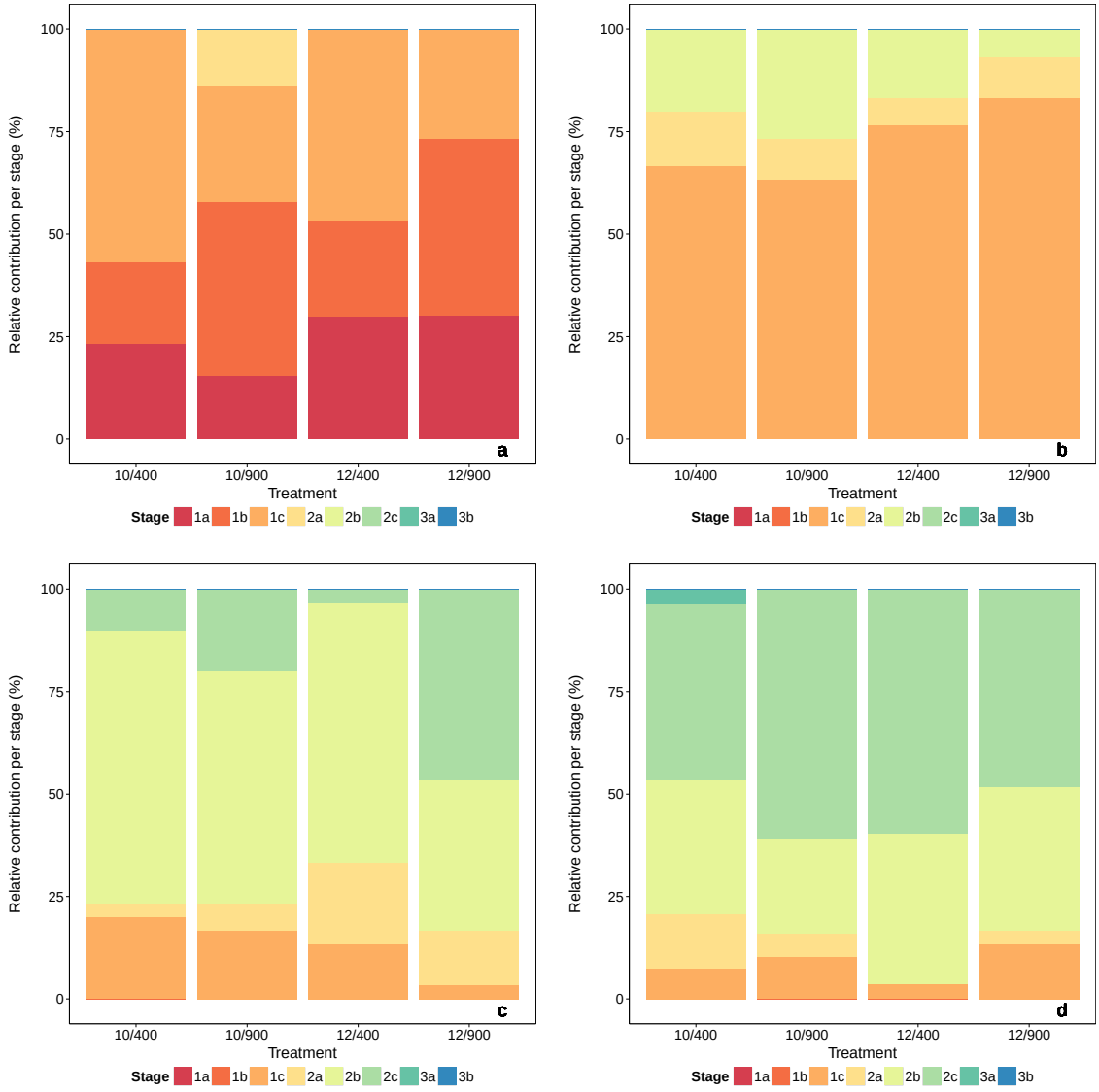

Figure 2.9: Relative contribution per developmental stage and treatment for selected sampling days: (a) 2 DPH, (b) 12 DPH, (c) 22 DPH and (d) 32 DPH.

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Indirect food web effects of ocean acidification increase herring larval survival

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Abstract

Ocean acidification, the decrease in seawater pH due to rising CO_2 concentrations, has been shown to decrease survival in early life stages and thus recruitment of fish populations including commercially important species (Baumann et al., 2011; Stiasny et al., 2016; Munday et al., 2015). Thus far, studies focused on direct physiological impacts of CO_2, ignoring indirect effects via food web interactions. Here we report from the most comprehensive ocean acidification experiment to date, an in situ mesocosm experiment with 50 m^3 enclosures that was run for 113 days. Within a complete pelagic food web, Atlantic herring larvae were exposed to realistic end-of-the-century ocean acidification ($\sim$760 $\mu\mathrm{atm}$ $p\mathrm{CO}_2$). Our findings show that the survival of herring larvae was significantly increased by 19 ± 2 % in the ocean acidification scenario. A comprehensive analysis of the plankton community revealed that the herring larvae benefitted from CO_2-driven increases in primary production, which in turn resulted in higher availability of zooplankton prey. Ocean acidification can thus alter trophic cascades and result in higher fish larvae survival if primary production is stimulated. Such effects complement earlier negative direct effects shown in laboratory experiments owing solely to increased $p\mathrm{CO}_2$ and which cannot be detected under constant food. These findings demonstrate the need for a better understanding of the interplay between physiological responses and food-web effects before we can even predict the direction of change in fish recruitment in a high CO_2 ocean.

3.1 Main text

There is increasing evidence that ocean acidification (OA), the decrease in seawater pH due to rising atmospheric pCO_2, can impair the development of early life stages of fish (Howes et al., 2015; Fabry et al., 2008; Wittmann and Pörtner, 2013). The survival of fish larvae represents a critical bottleneck for recruitment and fish stock development and is thus of particular importance for commercially exploited species (Haigh et al., 2015; Houde, 2008). Only few studies have investigated the direct physiological responses of fish larval survival as one important component of population growth to projected increases in atmospheric pCO_2. While ocean acidification decreased survival of the larvae of Atlantic silverside and Atlantic cod exposed to $\sim$1100 μatm pCO_2, no effect was detected in yellowtail kingfish larvae at $\sim$880 μatm pCO_2(Baumann et al., 2011; Stiasny et al., 2016; Munday et al., 2015). Notwithstanding these primary CO_2 effects, next to nothing is known how OA plays out in a food-web context. Since increased pCO_2 is expected to fertilize many but not all phytoplankton groups, we could envisage that larval survival may actually be enhanced if food is limited under ambient CO_2 but not under CO_2 enrichment (Sokolova et al., 2012; Thomsen et al., 2013).

Hence, we set-up a large mesocosm experiment, aiming at simulating a fully functional pelagic ecosystem to assess how CO_2 levels expected by the end of the century affect fish larval survival. We enclosed the endemic plankton community from the Gullmarsfjord, Sweden in ten mesocosms ($\sim$50m^3), to test for the combined direct physiological and indirect food web effects on the plankton community and herring larvae (Fig. 3.1). Five mesocosms were left untreated as controls and the remaining five were set to elevated CO_2 levels ($\sim$760 μatm pCO_2). The experiment lasted for 113 days, from March 7^{th} until June 28^{th} 2013, and the enclosed water column was sampled regularly for a comprehensive set of physical, chemical, and biological parameters, e.g. carbonate chemistry, nutrient concentrations, and phyto- and zooplankton abundances(Bach et al. 2016). Herring eggs were added into all mesocosms during the onset of the second bloom (April, 22^{nd}, after fertilization of the eggs on April, 18^{th})(Fig. 3.2. From hatch on May 11^{th} until final sampling on June 21^{st} (41 days post hatch) herring larvae lived and fed exclusively on the organisms in the mesocosms, so that potential CO_2 effects on the food web could be uplinked to growing herring larvae (also see Video (Sswat et al., 2016)). Survival rates were calculated based on the initial number of hatched larvae, dead larvae collected in the sediment traps over time, and number of survivors at the end of the experiment, assuming no loss from predation. Herring larval survival was split into two phases, differing in mortality over time (Fig. 3.3). The first phase (P1) lasted for 16 days post hatch until May 27^{th}, including the critical first feeding period (Cushing, 1990; Houde, 2008), and was characterized by high mortality. The second phase (P2), with comparatively low mortality, lasted from May 27th until June 21^{st}, the last day of sampling for fish larvae. Under elevated CO_2 levels herring larvae experienced on average 19 $\pm$ 2% higher survival than in mesocosms with ambient CO_2 levels (Cox-ph-model, $p<0.001$, E=0.81, sd=0.02) (Fig. 3). This difference in survival between CO_2 treatments was already visible after the first phase (t.test, $p<0.05$, F=-2.53). Survival in the first phase significantly correlated with prey abundances, i.e. particles in the size range of 100-300 μm and the sum of nauplii and copepodite abundances (Fig. 3.4), suggesting that prey availability was limiting and survival of herring larvae. No significant effect of elevated CO_2 levels on survival, nor a significant correlation with prey availability was found in the second phase alone (17-41 DPH). We further analyzed the data to substantiate a connection between primary production

and enhanced zooplankton availability. In the mesocosms, primary production and chlorophyll a, a proxy of phytoplankton biomass, were significantly higher under elevated CO_2 conditions ((Bach et al., 2016), Eberlein, pers. comm.). Higher production and biomass of primary producers at elevated CO_2 (Fig. 3.2) was significantly correlated to higher abundances of nauplii, copepodites and adult copepods (Fig. 3.3 b,c), suggesting that primary producers had previously been limited by primary production. The majority (>90%) of the adult copepods belonged to the species *Pseudocalanus acuspes* ((Algueró-Muñiz et al.), subm.). CO_2-stimulated primary production was transmitted through the food web all the way up to the top predators/herring larvae in this study. The significant difference in survival of herring larvae between CO_2-treatments may therefore be connected to differences in prey availability during the first days after hatch, which are a critical time for fish larvae due to the transition between endogenous energy supply and exogenous feeding mode (Houde, 2008). During this developmental phase a sufficient abundance of suitable prey items is needed to initiate successful feeding (Kiørboe et al., 1985). To sum up, the observed differences in herring larval survival among CO_2 treatments is completely consistent with measured prey abundances that were a suitable size range (Fig. 3.4) The decline in abundances in the different copepod stages (Fig. 3.3 b,c) reflects the subsequent size-switch of developing herring larvae to larger prey items (Checkley, 1982; Denis et al., 2016). Prey population decline is most likely caused by predation by herring larvae, which is additional evidence for the trophic linkage as the ecological mechanism to explain herring larvae performance.

These findings indicate that fish larvae benefitted indirectly from ocean acidification via food availability. Under OA, elevated CO_2 levels enhanced primary productivity and biomass transfer up the food web increased prey availability for fish larvae. Clearly, the food-web effect on fish larvae depends on the OA response of primary production in the first place. Thus, the potential effects of OA on primary productivity and trophic transfer efficiency to fish larvae could differ substantially depending on plankton community composition and additional environmental factors. Occurrence and interactions in the plankton community can severely affect survival and recruitment in fish (Cushing, 1990; Houde, 2008). Herring larvae were shown to be tolerant to moderate CO_2 levels projected for the end of the century ((Sswat et al.), under review) and may therefore be primarily affected through changes in food availability and quality. How OA induced changes in prey availability might influence early larval stages of CO_2-sensitive fish species showing stronger direct physiological responses to OA (e.g. Atlantic cod *Gadus morhua*) still needs to be resolved. However, increased prey availability in response to OA as observed in our study may have the potential to mitigate direct negative impacts of OA on survival and recruitment also in these more OA sensitive species, a result lab studies based on physiology only have not been able to detect. These findings underline the importance of considering food web interactions and potential OA-induced trophic cascades for future ocean studies on early life stages in fish. Before we can predict whether fish recruitment will increase or decline in the future ocean a better understanding of the interaction between direct physiological and indirect food web effects is essential.

3.2 Materials and methods

Experimental set-up

The mesocosm CO_2 enrichment experiment took place from March 7^{th} till June 28^{th} 2013 in Gullmarsfjord, a sill fjord located on the Swedish west coast (58.26635° N, 11.47832° E). The endemic plankton community from the Gullmarsfjord was enclosed in ten mesocosms (Riebesell et al. 2013) and the development monitored for 113 days. Each mesocosm consisted of a floating frame holding a translucent polyurethane bag of 2 m in diameter and 17 m in length with a conical sediment trap of 2 m length attached at the bottom, yielding an enclosure volume of ∼50 m^3. CO_2 treatment levels were set to ambient and elevated conditions (∼380 μatm and ∼760 μatm pCO_2, respectively), with each treatment replicated five times. Regular sampling every two days was conducted for various parameters e.g. carbonate chemistry, inorganic nutrient and organic matter concentrations, and sediment fluxes. For further details on the study, e.g. CO_2 manipulation and community responses, see Bach et al. (2016). The chronology of major events for fish larval addition to the ten mesocosms is shown in Figure **??**.

Fertile herring were caught by a gillnet at a depth of ∼30 m on April 22^{nd}, 2013 in the Oslo Fjord, south off Søndre Kaholmen (59°40'2" N and 10°36'2" E), in collaboration with the Biological Station Drøbak (University of Oslo). To allow for genetic variation, the sticky eggs of 5 females were strip-spawned on each of 20 plastic plates and eggs were gently mixed with the sperm of 5 males, resulting in 1230 to 3691 fertilized eggs per plate. Fertilization was performed in the lab at two CO_2 levels (ambient pCO_2: ∼470 μatm, high pCO_2: ∼900 μatm), similar to the CO_2 levels in the mesocosms in that time (Bach et al., 2016), to include the possible effect of CO_2 on fertilization success and sperm motility. Before introduction into the mesocosms, egg-plates were kept in a flow-through tank at ambient (∼470 μatm) pCO_2, to synchronize time of hatching and hatching success (Franke and Clemmesen, 2011)(pers. comm. F. Dahlke).

On April 26th each mesocosm received two of the egg-plates with a total of 3517 to 5421 eggs, which relates to the developmental stages 4 and 5 for the eggs (Klinkhardt, 1996). The egg plates were kept in "egg-cages", spherical mesh-cages, which allowed for a protected environment with optimal water exchange. The egg-cages were kept at 3 m until May 1^{st} and 6 m depth until May 12^{th}. To check on development and estimate the time of hatching the egg-cages were shortly lifted out of the water every two days in all mesocosms. The hatched larvae could easily escape into the mesocosm through an opening in the egg-cages and were left inside of the mesocosms for two more days to allow for the maximal number of larvae hatching. Time of peak hatch, 0 DPH (days post hatch), was estimated for May 11^{th} based on optical inspection. The initial number of hatched larvae (1175 to 2031 larvae mesocosm-1) was calculated by comparing the counted abundances of eggs from photographs on each egg plate after fertilization and after hatch. The few fallen off eggs and dead larvae in the egg-cages were counted and subtracted from the numbers of hatched larvae from the photographs.

Abiotic factors such as salinity, temperature and oxygen concentration inside the mesocosms were measured every two days with a CTD (Sea and Sun Technologies) and were close to the natural conditions surrounding the mesocosms. Temperature fluctuated between 8.5 °C and 11.6 °C (May 11^{th} - June 8^{th}), followed by an increase in temperature to 15.5 °C on June 21^{st}. The

oxygen concentration depended on depth and time but stayed at ∼100 μmol/kg (unpubl.) and mean salinity slightly increased due to evaporation, from 29.3 at hatch to 29.4 at final sampling (Bach et al., 2016).

Sampling

Since herring larvae could not be caught in the regular zooplankton-nets, additional larval sampling was performed with light-traps (BellaMare US) taking advantage of the positive phototactic behavior of the larvae. In total <70 specimens per mesocosm were removed during the experiment by light traps. Sampled larvae were accounted for in the calculation of larval survival, described in the section on statistical analysis. Survivors were sampled on June 21^{st} (41 DPH) (Fig. 3.2) by carefully pulling a ring-net of 1000 μm mesh size through the full length of the enclosed water column, thereby sampling all remaining fish larvae from the mesocosms. For this purpose the net was attached to a "cleaning-ring" which is used to wipe the inner side of the mesocosms and thus has exactly the same diameter as the mesocosm bags (Riebesell et al., 2013). The net was lowered in a folded manner so that fish larvae were not caught on the way downward. By pulling a rope at the deepest position of the ring (last segment of the bag above the sediment trap), the net unfolded with the same diameter as the cleaning ring so that no fish could escape when pulling it upwards. Fish larvae were prevented from escaping into the sediment trap by releasing air bubbles at the lowest part of the trap via the sediment sampling tube. This was additionally verified by visually inspecting the sediment trap by lowering a camera connected to a monitor.

All sediment trap material was sampled every two days following (Boxhammer et al. 2016), which allowed us to determine the number of dead larvae per mesocosm. For this purpose sediment material was visually inspected in photo dishes prior to regular sediment processing. The material was gently screened with forceps and dead larvae were quantified. First sighting of dead larvae in the sediment material was on May 25^{th} (14 DPH), depicted by the fish bone in (Fig. 3.2). Before 14 DPH, the dense sediment material and small larval sizes made a visual detection unreliable. It is assumed that all dead fish ended up in the sediment trap, because no affective fish larvae predator was present. Small sizes of the hydromedusae found in the mesocosm and no detection of predation on herring larvae support the assumption (Purcell and Grover, 1990). All larvae classified dead and alive were incorporated into the survival analysis. Number of dead larvae until May 25^{th} (X), the time when first dead larvae were detected in the sediment material, was determined indirectly by the difference between number of hatched larvae (H), survivors at the end (E), sum of larvae sampled alive (A) and sum of dead larvae from the sediment between May 25^{th} and the end of the experiment (D):

$$\mathrm{X} = \mathrm{H} - (\mathrm{E} + \mathrm{A} + \mathrm{D}) \tag{3.1}$$

Here, X represents the sum of dead larvae between May 11^{th} and May 25^{th} and thus cannot be assigned to specific sampling days. For the statistical analysis, May 25^{th} was set as sampling day for this sum of dead larvae (X).

To assess the influence of prey abundances on larval survival, zooplankton samples from each mesocosm were collected every eight days. The preferred prey of herring larvae, the different life

stages of copepods (nauplii, copepodites and adult copepods) were quantified from an Apstein-net (55 μm, 17cm). The majority (>90%) of copepods belonged to the species Pseudocalanus acuspes. Particle abundances of different size classes were obtained from an image-based analysis of plankton samples ("ZooScan method"(Gorsky et al., 2010)), where a subsample from the regular zooplankton net hauls was scanned with a flat bed scanner and automatically categorized and size measured. Prey size spectra, in terms of particle size, between 100 and 300 μm were considered for the first survival phase and between 300 and 800 μm for the second survival phase. These assumptions are based on prey size spectra in relation to herring larval standard length from Hufnagl and Peck (2011). In terms of zooplankton groups the abundance of copepod nauplii and copepodites was included for the first phase, whereas nauplii, copepodites and adult copepods were considered as prey in the second phase (Checkley, 1982). A potential effect of other prey items from the community cannot be excluded but may be considered minimal, due to the dominating contribution of copepod species to the community. Additional correlative analyses between smaller size classes of prey (ciliates) and fish larval survival could not improve the relationship.

Statistical analysis

We applied a Cox proportional hazards model (Harrell, 2001) for the survival analysis over the whole period of the experiment. Here, the hazard value "E" represents the risk of a treatment group to die relative to a control group (E=1 by default), e.g. if E=0.8 for the treatment group, it would imply a 20% lower mean survival than the control group. For the survival analysis of the two separate phases, the survival at the end of the respective phase was calculated and checked for significance by one-way-ANOVA. The same values were used to check for Pearson's product-moment correlation between survival and particle abundances in the respective phases. For all statistical tests a p-value of 0.05 was considered as threshold of significance.

Ethical permit

The experiment was permitted by the Swedish Board of Agriculture '"Jordbruksverket" (ethical permission number 332-2012). Minimization of stress from treatment and handling assured welfare of herring larvae. Herring larvae were anaesthetized before handling using Tricaine methanesulfonate MS-222. The CO_2 concentrations used in this study are far below the lethal level and the species used, *Clupea harengus* is not endangered.

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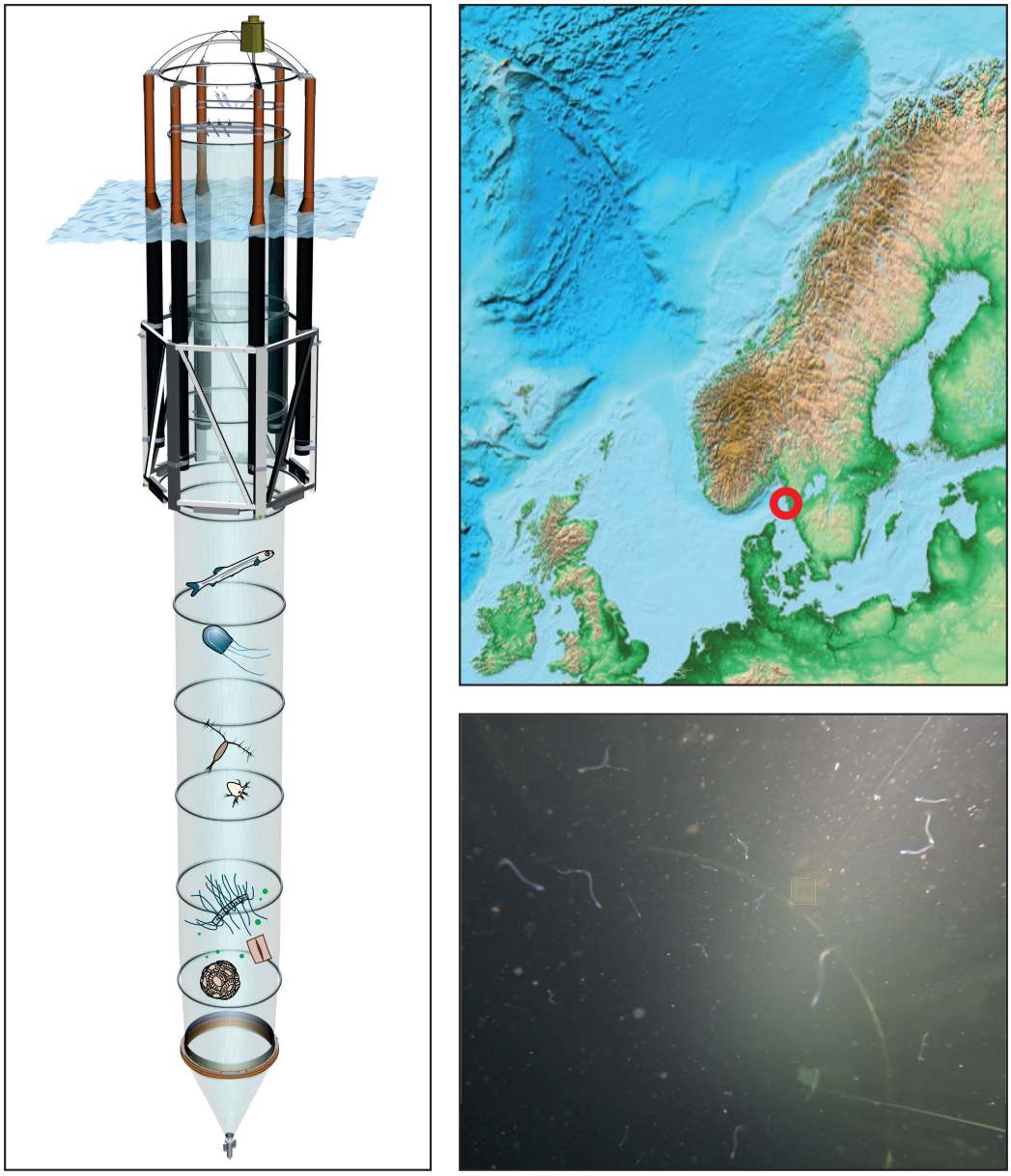

Figure 3.1: Schematic drawing of a mesocosm (left), the location area indicated by a red circle (upper right) and a frameshot of herring larvae in the mesocosm (lower left).

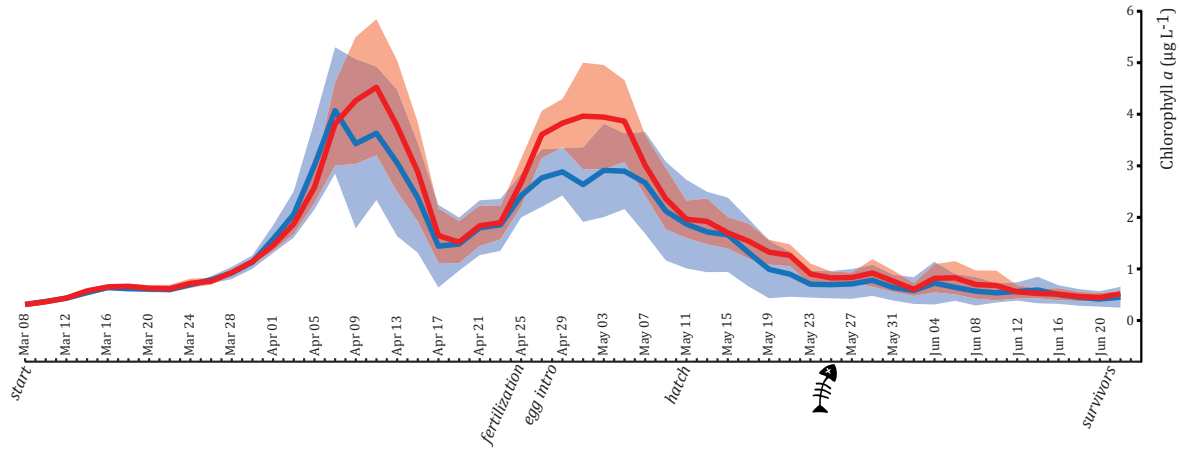

Figure 3.2: Experimental setup and chronology of major events: Mean chlorophyll a concentration over time (blue and red colors depict ambient and high CO_2-levels, respectively) and dates of introduction, hatch and final sampling of herring larvae. P1 and P2 depict the selected phases of survival, based on times of high and low mortality (P1 and P2, respectively)The fish bone depicts the start of two-day counts of dead larvae detected in the sediment trap.

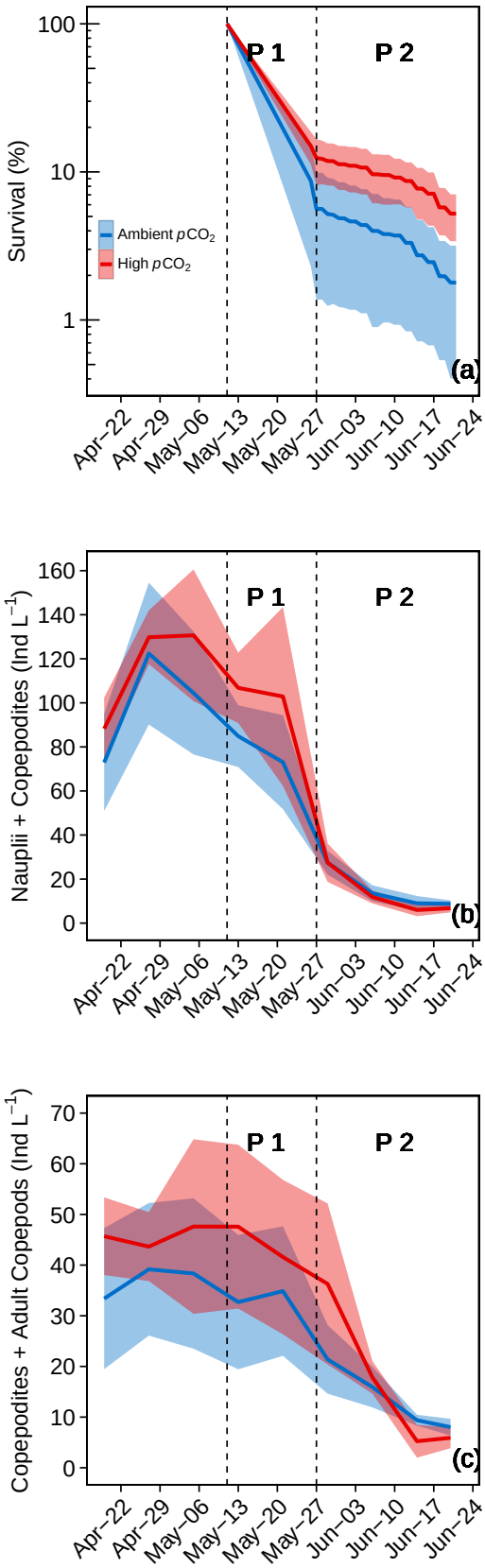

Figure 3.3: Development over time under ambient and high CO_2 treatments of (a) herring larval survival, (b) the sum of nauplii and copepodites abundances (ind L^{-1}) and (c) the sum of copepodites and adult copepods abundances (ind L^{-1}). The shaded area depicts the standard deviation around the mean. Dashed lines separate the two phases of survival, P1 and P2 relate to the time of high and comparatively low larval mortality, respectively.

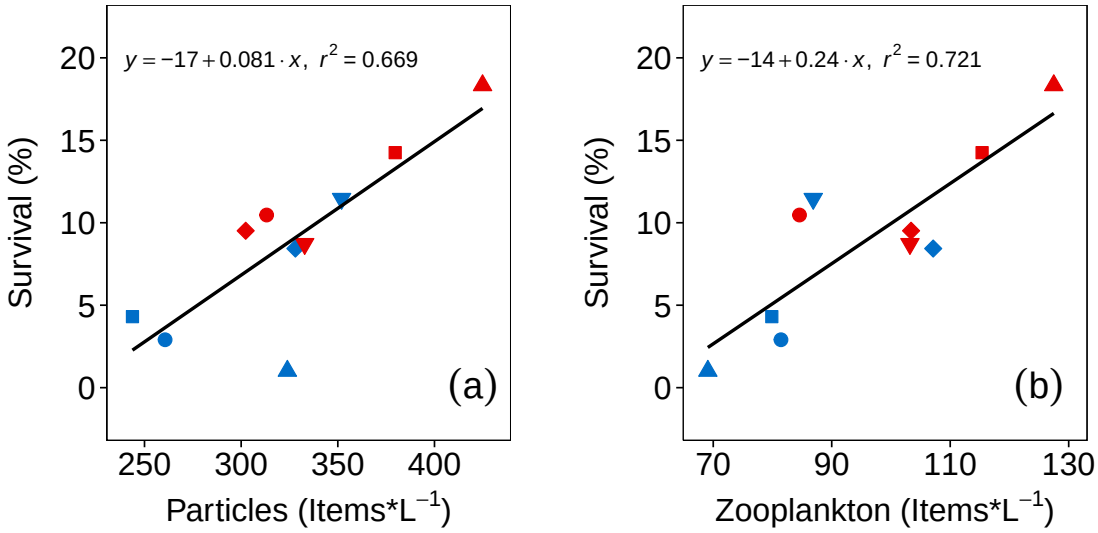

Figure 3.4: Relation between survival and the abundance of prey items. Survival in phase P1 (May 11^{th} - May 27^{th}) to (a) particles in a size range of 100-300 μm(L^{-1}) and (b) nauplii + copepodites (L^{-1}) on May 11^{th}. Blue and red colors depict ambient and high CO_2-levels, respectively.

4 | Manuscript III

Effect of ocean acidification on prey availability and growth performance of herring larvae in large scale mesocosms

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Abstract

Early life stages of commercially important fish species may face direct physiological and indirect food web effects of ocean acidification by rising CO_2 concentrations. We studied the combined direct physiological and indirect food web effects of ocean acidification on herring larvae inside pelagic mesocosms. A natural plankton community of the Gullmarsfjord, Sweden was enclosed in the Kiel Off-Shore Mesocosms for future Ocean Simulations (KOSMOS) for 104 days from March to June 2013 at ambient and projected end-of-the-century CO_2 levels ($\sim$760 μatm pCO_2). Eggs of Atlantic herring, *Clupea harengus* were introduced into the mesocosms end of April, where they hatched two weeks later in mid of May. The larvae developed inside the mesocosms for 41 days, feeding on prey organisms that experienced treatment CO_2 levels already for 63 days. Otolith increment width was used as a proxy for growth of the survivors during the course of the study. For selected phases otolith increment growth can be related to food availability and intra-species competition. Higher larval abundances in the elevated CO_2 treatment relate to a decreased amount of prey items per larvae and thus possibly lower growth. However, this did not lead to a significant difference between treatments in growth of the survivors at final sampling, resembled by instantaneous growth rate and growth in length and dry weight. The content of essential fatty acids in the larval herring was also unaffected by elevated CO_2 levels, suggesting no difference in food quality between treatments. Our results highlight knowledge gaps and underline the importance to incorporate indirect community effects such as prey availability and competition in ocean acidification studies.

4.1 Introduction

The strongest physiological direct effect of OA in fish is expected for the early developmental stages (Kikkawa et al., 2004; Brown and Sadler, 1989), since the gills, as main organs of osmoregulation, are not fully functional yet (Rombough, 1988). At elevated CO_2 concentrations energy requirements may be increased for osmoregulation but also for development of the respective organs (Pörtner et al., 2004). Effects of elevated CO_2 levels were studied for the early life stages of various fish species,. Little or no effects of CO_2 on egg and larval development, growth and behavior were reported for the larvae of some species, like orange clown-fish, mahi-mahi, walleye pollock, three-spined stickleback and Baltic cod (Munday et al., 2009; Bignami et al., 2014; Hurst, 2013; Schade et al., 2014; Frommel et al., 2012), while for other species impacts e.g. on organ structure and survival in Atlantic cod (Frommel et al., 2011; Stiasny et al., 2016), embryonic survival in two-spotted goby (Forsgren et al., 2013), and behavior in spiny damselfish and dolphinfish (Welch et al., 2014; Pimentel et al., 2014) were observed.

In Atlantic herring, effects of OA on life traits can differ between populations as shown for the Norwegian spring spawning (NSS) and the Baltic Sea population (BS) (Frommel et al., 2014; Bodenstein, 2012; Franke and Clemmesen, 2011; Maneja et al., 2014, 2015). These differences in response may be caused by adaptation to the seasonal variation and biological activity in coastal areas (Barrio et al., 2016). As a bottom spawning species, *C. harengus* and their early life stages may already encounter carbon dioxide levels higher than expected for the end of the century, especially in the Baltic Sea (Melzner et al., 2009; von Dewitz, 2012) nowadays. Negative OA effects in the BS population were observed on egg survival at pCO_2 levels of $\sim$4000 μatm (Bodenstein, 2012) and nutritional condition in terms of RNA/DNA ratio at pCO_2 levels of $\sim$1800 μatm (Franke and Clemmesen, 2011), while standard length, dry weight and hatching rate even at pCO_2 concentrations up to 4600 μatm seem to be unaffected. In older larvae of the NSS population pCO_2 levels >1800 μatm impacted larval growth, development, condition and tissue formation (Frommel et al., 2014) but had no effect on specific traits like swimming behavior and proteome structure at 4200 μatm pCO_2 (Maneja et al., 2014, 2015). At CO_2 levels expected for the end of the century (900 μatm pCO_2) herring larvae seem to be unaffected in terms of survival, growth and development ((Sswat et al., a), under review).

The question so far not being studied is how the potentially OA-induced changes in the food web, especially regarding prey availability, will affect growth of fish larvae. The preferred prey for the larvae of Atlantic herring, a commercially important fish species in the North Atlantic, are the different life stages of calanoid copepods (Checkley, 1982; Cohen and Lough, 1983). Also protists, like diatoms, dinoflagellates and ciliates, may serve as an additional food source in the first days after hatching (Denis et al., 2016; Montagnes et al., 2010; Busch, 1996) and can promote early feeding (Illing et al., 2015). Trophic upgrading, the increase in food quality by an intermediary trophic level, from microplankton to copepods may also occur (Klein Breteler et al., 1999), which in turn could cause better somatic growth of the larvae due to better fatty acid profiles in the food (Beckmann, 2015). Therefore OA induced changes in prey items will likely affect herring larval growth.

In larval and juvenile fish, growth rate is not only influenced by prey quantity but also by the biochemical composition (food quality), e.g. the content of essential fatty acids (EFA) (Watan-

abe, 1982; Koven et al., 1993; Bell et al., 1995; Cutts et al., 2006; Paulsen et al., 2013a). Earlier studies showed that the amount of EFA and their relative compositions can influence fish larval growth and survival (St. John, 2001; Copeman et al., 2002). Since fish larvae cannot produce EFAs themselves, they fully depend on the transfer of EFAs from their prey (Fraser and Sargent, 1989; Navarro et al., 1993; Rossi et al., 2006). In a large scale mesocosm community approach a reduction in the relative PUFA (polyunsaturated fatty acid) content of the dominant copepod was most likely caused by an OA-induced shift in phytoplankton community composition (Bermúdez et al., 2016). OA induced changes in the phytoplankton composition can be uplinked to higher trophic levels and may thus indirectly influence fish larval somatic growth (Rossoll et al., 2012; Bermúdez et al., 2015; Dalsgaard et al., 2003; Paulsen et al., 2013b; St. John, 2001).

A well-studied tool to reflect the growth patterns in larval and juvenile fish is the analysis of the microstructure and increment width of otoliths (earstones). Otoliths can be used as growth recorders, where otolith growth reflects somatic growth (Campana and Jones, 1992). Otolith analyses can thus be used to detect differences in growth over time and relate these for example to changes in prey availability, as shown for larval herring in land based mesocosms (Folkvord et al., 1997).

4.2 Materials and methods

Experimental set-up

The simultaneous direct physiological and indirect food web effect of elevated CO_2 levels on herring larval performance was studied in a close to natural mesocosm experiment using ten pelagic mesocosms enclosing a water body of $\sim$50 m^3 containing an endemic plankton community of the Gullmarsfjord, Sweden. One half of the mesocosms was manipulated to elevated CO_2 levels ($\sim$760 μatm pCO_2), while the other half served as untreated controls. The development of the plankton community was regularly monitored in relation to carbonate chemistry, nutrients, phytoplankton abundance and zooplankton abundance. Details on the development of the planktonic community can be found in Bach et al. (2016). The introduction of herring eggs and larvae into the mesocosms, is described in detail in the "Materials and Methods" section of (Sswat et al. (b), in prep.). Briefly, eggs from adult herring caught in the Oslo Fjord were stripped on plastic plates. These "egg-plates" were introduced into the mesocosms, where larvae hatched on day t63 and exclusively fed on the organisms from the enclosed community. To relate days of the experiment (t) to the age of herring larvae in days post hatch (DPH), both units are presented.

Sampling

Fish larvae cannot be caught by regular zooplankton-nets (ø: 20cm, mesh size: 55 μm, Apstein net) taken once a week due to avoidance. Thus, in between sampling of alive larvae was performed by light-traps (height: 114.3 cm, ø: 45.7 cm, mesh size: 500 μm, BellaMare US), taking benefit of the larvae's positive phototactic response (Woodhead and Woodhead, 1955; Catalán et al., 2011). This technique is considered to be the least invasive larval sampling technique within a mesocosms system. The light traps were lowered to a depth of 5-10m for 8 hours per night (22.00-7.00) on several days of the experiment (Fig. 4.1, Table 4.1). As the amount of herring larvae caught by light traps was very low and highly variable between mesocosms after day t73 (10 DPH), these larvae were excluded from further analysis. In total very few specimens were removed during the experiment by light traps.

Survivors were sampled on t104 (41 DPH) by carefully pulling a ring-net through the whole water column of the mesocosm (Fig. 4.1, Table 4.1). For this purpose the net was attached to a "cleaning-ring" which is used to wipe the inner side of the mesocosms and thus has exactly the same diameter as the mesocosm bags (Riebesell et al., 2013). The net was lowered in a folded manner so that fish larvae were not caught on the way downward. By pulling a rope at the deepest position in the mesocosm (last segment of the bag above the sediment trap), the net unfolded with the same diameter as the cleaning ring so that no fish could escape when pulling it upwards.

The larvae caught by light-trap and ring-net were transported to the field station and directly processed in the laboratory. Larvae were anaesthetized with Tricaine mesylate (MS-222) prior to handling. Ten randomly chosen larvae were photographed per mesocosm using a digital camera attached to a stereomicroscope (Olympus SZX 7 with Olympus DP 26 Camera and Olympus Stream Essentials Software) and stored individually at -80°C. The remaining larvae were pooled

(5-10 larvae per plastic cup) and stored at -80°C for further analysis (Table 4.1). Analysis on larval length, dry weight, RNA:DNA ratio, otoliths and fatty acid composition were conducted at GEOMAR, Kiel and AWI, Helgoland from frozen and ethanol-preserved samples. Multiple analyses were performed on single larvae due to the low sample sizes (Fig. 4.2).

Analyses

Growth, RNA/DNA and fatty acid analysis

Larval standard length (SL) was measured with the open source software Image J (Abràmoff et al., 2004) from the calibrated pictures of each larva. Larval dry weight was determined to the nearest 0.1 μg (Sartorius, Secura 224 and SC2) after freeze-drying for 18 h at -51°C until weight remained constant (Christ Alpha 1-4 freezedryer). Individual larval growth in length (G_{SL}) and dry weight (G_{DW}) per day was calculated by the following formula for each larva (4.2, **??**, where SL_{first} and DW_{first} are the average SL and DW in the first days after hatch:

$$G_{SL} = (SL_{final} - SL_{first})/(DPH_{final} - (DPH_{first}) \tag{4.1}$$

and

$$G_{DW} = (\ln DW_{final} - \ln DW_{first})/(DPH_{final} - (DPH_{first}) \tag{4.2}$$

SL_{final} and DW_{final} is the individual SL and DW at final sampling (t104). A growth period of 37 days was assumed based on the average difference between sampling in the first days after hatch and final sampling (t104)(Table 4.1).

The larvae selected for fatty acid (FA) analysis were stored at -80°C until being transported on dry ice to the AWI, Helgoland (less than 8 hours). FA analyses were carried out according to a modified method described by (Malzahn et al., 2007). The following fatty acids were selected for statistical analysis due to their importance for fish larval growth and development (Sargent et al., 1993, 1997): DHA (Docosahexaenoic acid), EPA (Eicosapentaenoic acid) and the cumulative content of all polyunsaturated fatty acids (PUFA). The content of the essential fatty acid ARA (Arachinoic acid) was at or below the detection limit and therefore excluded from further analysis.

After the extraction of fatty acids, the "defatted" larvae as well as those not used for FA analysis were rehydrated for otolith dissection and immediately frozen afterwards for lyophilisation (freeze-drying). The lyophilized larvae were analyzed for the content of nucleic acids, according to the protocol described in (Malzahn et al., 2003). Some of the larvae used for the analysis of nucleic acid content were not used in the fatty acid analysis described above. A comparison between larvae analyzed by the classical approach (Clemmesen, 1988, 1994) and the adapted approach (Malzahn et al., 2007) showed no significant difference between the approaches (Paulsen et al., 2017).

The ratio of RNA to DNA was standardized to sRD according to (Caldarone et al., 2006) and individual weight-specific growth rate per day (G_i) was calculated, using the growth model given in (Buckley et al., 2008), which takes into account the activity of RNA at different temperatures. For this purpose temperature in the mesocosms from the day of sampling was considered.

Otolith analysis

We used the largest otolith, the sagitta for the following analysis. Damaged or "unreadable" otoliths were excluded from further analysis leaving 29 otoliths from the survivors at the end of the experiment. From each sagitta, the longest radius from the center to the last ring, the radius from the center to the first ring, as well as the number and width (IWI) of the increments were determined under a camera-mounted microscope-computer system (Q Imaging MicroPublisher 3.3 mounted with Leitz Laborlux S) with the software ImagePro (Image Pro Insight v. 8.0.3, Media Cybernetics Inc.). An increment was determined as the distance between two rings (dark) and the width of the increment was measured on the longest radius of each otolith. Rings were adopted from the vicinity when invisible on the longest radius. Inter-reader comparisons and multiple readings of otoliths from larvae with a known age increased the precision of the increment determination. In herring, increment number does not reflect age in days post hatch. The first ring, also termed "first check", is most likely formed at the start of external feeding (Geffen, 1982; Moksness and Fossum, 1992). The time between hatch and the formation of the first ring, 9 days, found in this study is within the range reported for herring in the literature (Geffen, 1982). This calculation is based on those larvae caught alive at the end of the experiment, since both the number of increments as well as their age in DPH is known.

Prey availability

To relate otolith growth to prey availability different time phases were chosen. Each phase represents the mean increment width of 8 days compared to the average abundance of two consecutive zooplankton samplings, which are also 8 days apart from each other (Fig. 4.1). A lag in response of four days was assumed between prey abundance and IWI pattern in the larvae (Peck et al., 2015). Phase I (< 14 DPH) was neglected to minimize irregularities in determining first increment formation after the first check. Two different possibilities to describe prey availability resulted from the zooplankton sampling (prey categories). The first prey category (zooplankton) considers the abundances of different copepod life stages and ciliates. The second category (particles) includes these organisms but also involves detrital particles in the given size classes (Table 4.3). Based on the literature preferred prey items and size spectra were selected to relate larval herring growth to prey availability (Checkley, 1982; Hufnagl and Peck, 2011; Cohen and Lough, 1983). Average herring larval abundance in each phase, was calculated from larval survival rates in (Sswat et al. (b), in prep.). Herring larval abundances were furthermore used to calculate the amount of prey items available per individual larvae.

Statistical analysis

The statistical analyses were performed with the respective RStudio packages (Team, 2015) for all parameters taken at final sampling. A one-way-ANOVA (t-test) approach was performed to analyze treatment effects on growth in length, content of DHA, EPA and PUFA and the ratio DHA:EPA, after checking for homogeneity of variances. We used the non-parametric Wilcox-Mann-Whitney-test in case of heterogeneity of variance to test for differences in growth of dry weight and the instantaneous growth rate G_i.

We used a general linear model (ANCOVA) to analyze CO_2-effects and those of other contin-

uous variables on the otolith increment width. In cases of heterogeneity of variance, we applied a generalized-least squares-model and compared the results from this analysis to those from the concurrent general linear model (Table 4.4). The best model was considered to be the one with the smallest Akaike Information Criterion, which was insignificantly different to the more complex models. The significance level for all statistical analysis was set to $p< 0.05$.

Ethical permit

The experiment was performed under the ethical permission (number 332-2012 issued by the Swedish Board of Agriculture "Jordbruksverket"). Animal welfare was assured by minimization of stress from handling and treatment. Specimens were therefore anaesthetized before handling using Tricaine methanesulfonate MS-222. The CO_2 concentrations used in this study are far below the lethal level and the species used is not endangered.

4.3 Results

Growth rate and essential fatty acid content

Growth in terms of dry weight, standard length and instantaneous growth rate showed high variability within, but no significant difference between CO_2 treatment (Fig. 4.3). In general variability was higher in the ambient than in the high CO_2 treatment (Table 4.4). Elevated CO_2 concentrations had no significant effect on neither the content of the essential fatty acids DHA- and EPA-content and their ratio (Table 4.4), nor for the cumulative content of all polyunsaturated fatty acids (PUFA) in the final larval sample (Fig. 4.4). No significant correlation was found between the content of DHA and EPA to any of the growth parameters.

Growth determined from otolith increment width analyses

Larval fish size and otolith size of herring larvae are significantly linearly related with the slope of the relationship not affected by CO_2 treatment (linear model: Otolith size $\sim$ Fish size, $F_{1,39}$=155.43, $p < 0.001$). Therefore data were combined to describe the relation between larval size and otolith size in this study in the following formula 4.3, based on those larvae with known size (SL) and otolith data, where OR is the otolith radius (Fig. 4.5):

$$\mathrm{SL} = 10.10 + 0.11 * \mathrm{OR} \tag{4.3}$$

To compute larval length at a specific age, OR at the respective age needs to be calculated from the radius of the first check plus the cumulative increment width of the larvae at the respective age.

The linear regression models using otolith increment width and prey fields of the survivors show differences between the selected phases (Table 4.5). In phases III and IV increment width is significantly lower in larvae from the high CO_2 treatment (Fig. 4.6). In phase III and IV also higher larval abundances are found in the high CO_2 treatment. Due to this correlation lower

increment width cannot be assigned specifically to the direct CO_2 effect or the indirect effect of higher larval abundances in these phases. In phase IV increment width is also positively related to increasing prey abundance (Fig. 4.7) independently from the prey category. In phase III particle abundance and biomass are positively correlated to increment width, while zooplankton abundance shows a negative correlation. In both phases III and IV the abundance of hydromedusae significantly improves the respective linear models. A positive relation between hydromedusae abundance and herring larval growth in increment width was detected for the respective phases. For phase II the selected covariates only explain the observed differences in otolith increment width to a minor extent ($R^2 < 0.25$). For phase V no model could be fitted with the selected covariates.

Herring larval abundance is decreasing over time and with differences between treatments, as more larvae (individuals m^{-3}) are found in the high CO_2 treatment (Fig. 4.8a). Larval abundances per treatment decline from 2.0 ± 0.9 and 3.3 ± 1.0 larvae m^{-3} for the ambient and high CO_2 treatment respectively in phase II, to 0.5 ± 0.2 and 1.0 ± 0.3 larvae m^{-3} for the ambient and high CO_2 treatment, respectively in phase V. The amount of prey items (nauplii and copepodites) per individual larva (items * $larvae^{-1}$) is on the average higher under ambient CO_2 conditions (Fig. 4.8b).

4.4 Discussion

Otoliths as growth recorders

The novelty of this study was to analyze the effect of future OA on herring larval growth in a close-to-natural plankton community within large scale mesocosms. Growth in length and weight as well as the instantaneous growth rate showed no significant difference between CO_2 treatments at the end of this study period. Since it was not possible to obtain larval samples during the experiment due to logistic constraints the effect of prey abundances on larval growth in length and weight could not be directly assessed. Otoliths increment width analyses can be used to tackle that problem as they serve as "growth-recorders". Otolith growth reflects somatic growth e.g. shown by Geffen (1982); Folkvord et al. (2000) and is supported by the correlation between otolith radius and larval length in our study. Thus no apparent decoupling between otolith and somatic growth needs to be considered as found at low feeding regimes (Barber and Jenkins, 2001; Baumann et al., 2005).

Focusing on differences in otolith increment width of the survivors therefore gives the opportunity to test how larval growth is influenced by different covariates, such as CO_2 treatment, prey abundance and the abundance of hydromedusae retrospectively. The covariates considered in the statistical analyses could only explain the observed differences in increment width to a minor extent. A better temporal resolution of prey availability and a higher sample size of the otoliths may help in future studies to evaluate these relationships more precisely. Still it is encouraging that analysis of otolith increment width can serve as useful tools to interpret differences in fish larval growth over time when direct estimates during the study are difficult to achieve.

Effect of prey availability

Prey abundance of particles in the preferred size range and preferred prey organisms have a significant positive influence on increment width and thus growth between 14 and 38 DPH (Phases II, III, IV). This result is not surprising and the relationship between food availability and growth is widely known (e.g. (Pepin et al., 2015) and references therein). The question arising is in fact if prey abundances and thus growth can be linked to OA? Otolith increment analyses for the specified phases (Phases, II, II; IV) are not conclusive. Some data suggest a negative CO_2 effect and at the same time a positive effect of prey availability. A negative physiological effect of CO_2 in herring larvae cannot be excluded, but seems rather unlikely, because CO_2 levels herring larvae were shown to be tolerant to pCO_2 levels $\sim$900 μatm ((Sswat et al., a), under review). Furthermore no significant negative CO_2 effect was detectable at final sampling for other parameters, such as growth and essential fatty acid content.

Effect of predation

In fact, a seemingly "negative CO_2 effect" leading to smaller increment width in phases III and IV might display the effect of competition for food between larvae and thereby reduced growth. In the respective phases, between 16 and 41 DPH, almost twice as many larvae lived in the high CO_2 compared to the ambient treatment, for details see Sswat et al. (b)(in prep.). Hence a stronger grazing pressure can be expected from higher abundance of herring larvae in the high CO_2 treatment. This effect of competition was also described for herring larvae in big enclosures by (Gamble et al., 1985). An indication of grazing by herring larvae might be found in the subsequent decrease of ciliate, nauplii and copepodite abundances after herring larvae hatched, in synchrony with the natural succession of zooplankton (Horn et al., 2016; Algueró-Muñiz et al.). For example, herring larvae can be assumed to feed preferably on copepodites in phase IV. Mean copepodite abundance (cop m^{-3}) declines in phase IV between t89 (26 DPH) and t97 (34 DPH) from $\sim$7900 (cop m^{-3}) to $\sim$5800 (cop m^{-3}) in the ambient, and from $\sim$8600 (cop m^{-3}) to $\sim$3800 (cop m^{-3}) in the high CO_2 treatment. This relates to a daily decline of 260 and 600 (cop m^{-3})*day^{-1}. Based on the average herring larval abundance in this phase and daily prey ingestions rates for one larva of 100-400 (prey items*larva^{-1})*day^{-1}) given in Bang et al. (2007) predation rates can be calculated. An estimated loss in copepodite abundance from herring larval predation may thus be in the range of 100-400 (cop*m^{-3})*day^{-1} and 200-800 (cop*m^{-3})*day^{-1} for the ambient and high CO_2 treatment, respectively. Therefore herring larvae had the potential to significantly decimate copepodite abundance in the mesocosms.

Effect of prey quality

Fish larval growth and survival can be affected by changes in prey abundances but also by alterations in the quality of prey organisms e.g. represented by the content of essential fatty acids (EFA), like DHA and EPA (Nunn et al., 2012; Watanabe, 1982; Koven et al., 1993; Bell et al., 1995). Differences in EFA composition may result from different EFA profiles of their prey, as fish larvae cannot synthesize EFA on their own (Fraser and Sargent, 1989; Navarro et al., 1993; Rossi et al., 2006). But OA might affect mechanism to store and process EFA in fish larvae. OA has been found to affect various fatty acids in larval red drum, *Sciaenops ocella-*

tus (Diaz-Gil et al., 2015), while Murray et al. (2016) detected only minor effects on fatty acid profiles in Atlantic silverside, Menidia menidia, both studies were performed at ∼2100 μatm pCO$_2$ compared to 760 μatm pCO$_2$ in this study. Elevated CO$_2$ concentrations were found to have a negative effect on EFA content in phytoplankton with consequences for the consumers, like copepods (Rossoll et al., 2012; Bermúdez et al., 2016, 2015). If OA induced changes in the food web affected orey quality of herring larvae in this study remains speculative, since data on the EFA composition of potential prey organisms are not available. The EFA composition of the herring larvae at the end of the experiment was unaffected by CO$_2$ treatment, suggesting no effect of OA on food quality in the last part of the study. If effects on fatty acid composition influenced larvae during the first part of the study cannot be assessed due to the limited data available. Growth and DHA content of fish larval are reported to be positively correlated (St. John, 2001; Cutts et al., 2006; Paulsen et al., 2013a), but this was not detected in our study. One explanation may be a sufficient supply in DHA for the larvae via the food web, as DHA content of larvae in this study lie in the upper range compared to larval herring from the Baltic Sea (Paulsen et al., 2013a). Another reason could be that high prey quantities substituted for low quality prey as suggested by Paulsen et al. (2013b). As the same study also found the substitution of low prey quantities by high quality, both aspects of prey availability should be in the focus of future studies.

Conclusion

Concluding, even in the well-determined ecosystem of the Kiel-Offshore-Mesocosms-for-future-Ocean-Simulations (KOSMOS) herring larval growth cannot fully be explained by the assessed parameters and shows the difficulties in estimating fish larval growth in a natural system. The positive cascading effect via the food web induced by increased CO$_2$ levels found for herring larval survival in the same experiment did not positively affect herring larval growth. This might be related to an increased predation pressure and thus competition between herring larvae, caused by higher survival rates in the high CO$_2$ treatment. Though direct and indirect effects on herring larval performance cannot fully be distinguished in this study, a direct physiological effect of CO$_2$ seems to be unlikely. This highlights the importance to incorporate indirect effects of CO$_2$such as prey availability and competition in future ocean acidification studies.

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Tables and figures

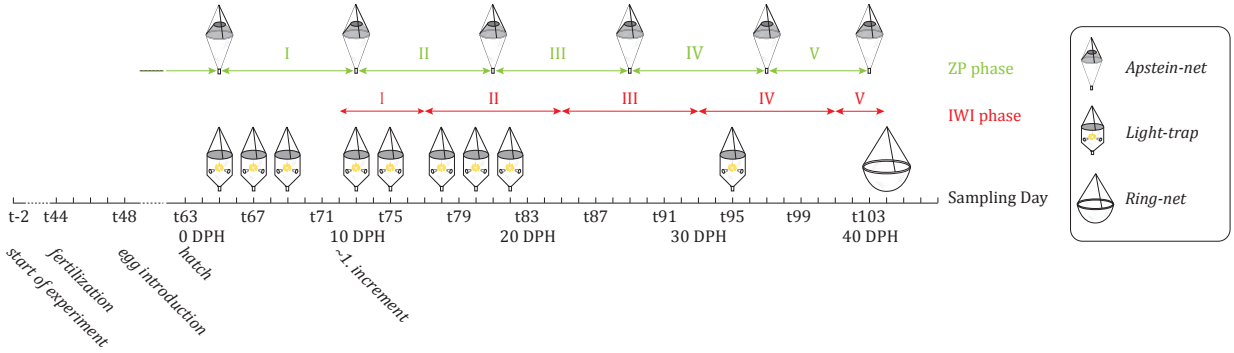

Figure 4.1: Overview of activities in the mesocosm experiment with a focus on herring larval sampling. Light traps and the ring net depict sampling of herring larvae. Apstein nets illustrate the time of sampling for zooplankton. The averaged increment width of the herring larvae otoliths for certain phases (IWI phase) relate to the respective phases of the averaged abundance of zooplankton (ZP phase).

Table 4.1: Number of larvae caught per sampling, mesocosm and treatment by light traps and ring net (day of the experiment (t) and age of the larvae in days post hatch (DPH).

Day (t)	DPH	Mesokosmos	CO_2-Treatment	Sampling method	Larvae
65	2	2	High	Light trap	3
65	2	5	Ambient	Light trap	2
65	2	6	High	Light trap	1
65	2	9	Ambient	Light trap	2
65	2	10	Ambient	Light trap	23
67	4	2	High	Light trap	23
67	4	3	Ambient	Light trap	4
67	4	4	High	Light trap	30
67	4	5	Ambient	Light trap	17
67	4	6	High	Light trap	63
67	4	7	High	Light trap	17
67	4	8	High	Light trap	28
67	4	9	Ambient	Light trap	26
67	4	10	Ambient	Light trap	19
69	6	3	Ambient	Light trap	1
73	10	1	Ambient	Light trap	1
73	10	2	High	Light trap	1
73	10	3	Ambient	Light trap	2
75	12	1	Ambient	Light trap	2
78	15	4	High	Light trap	9
80	17	6	High	Light trap	1
80	17	7	High	Light trap	1
80	17	9	Ambient	Light trap	5
82	19	5	Ambient	Light trap	1
95	32	6	High	Light trap	1
95	32	8	High	Light trap	1

Table 4.2: Table 4.1, continued: Number of larvae caught per sampling, mesocosm and treatment by light traps and ring net (day of the experiment (t) and age of the larvae in days post hatch (DPH).

t	DPH	Mesokosmos	CO_2-Treatment	Sampling method	Larvae
104	41	1	Ambient	Ring net	13
104	41	2	High	Ring net	28
104	41	3	Ambient	Ring net	2
104	41	4	High	Ring net	86
104	41	5	Ambient	Ring net	12
104	41	6	High	Ring net	48
104	41	7	High	Ring net	29
104	41	8	High	Ring net	52
104	41	9	Ambient	Ring net	31
104	41	10	Ambient	Ring net	42

Table 4.3: Summary of larval length, age, phase and the considered prey items used to relate otolith increment width to prey abundance. Prey items are differentiated into two categories: the first is based on zooplankton abundance while the second considers the abundance of particles in the selected size range.

Larval length	Age	Phase	Prey category	
			zooplankton class	particle size
12 - 14 mm	15 - 22 DPH	II	Nau + Cil	100 - 400 μm
14 - 17 mm	23 - 30 DPH	III	Nau + Cop	100 - 600 μm
17 - 21 mm	31 - 38 DPH	IV	Cop + Adu	100 - 800 μm
> 21 mm	39 - 42 DPH	V	Cop + Adu	100 - 800 μm

Table 4.4: Statistical analyses on the effect of CO_2treatment at the time of final sampling (41 DPH). For each parameter the respective p values are given as well as F- and W-values for t-test and Wilcox-Mann-Whitney-test (Wilcox), respectively. The stated parameters have the following abbreviations: growth in length = G_{SL}, growth in weight = G_{DW}, instantaneous growth rate G_i, docosahexaenic acid = DHA, eicosapentaenoic acid = EPA, and poly-unsatured fatty acids = PUFA.

Parameter	**Analysis**	**F- / W-value**	**p**
G_{SL}	t-test	0.40	0.70
G_{DW}	Wilcox	15.00	0.69
G_i	Wilcox	14.50	0.75
DHA	t-test	-0.95	0.37
EPA	t-test	1.01	0.35
DHA:EPA	t-test	-1.07	0.32
PUFA	t-test	-0.65	0.54

Table 4.5: Results of statistical analysis on otolith increment for the different phases with respect to the prey category. The coefficient of determination (R^2) for each general linear model is given, with the respective p- and F-values of the covariates. The factors listed are additive for each linear model (CO_2 = CO_2-Treatment, Nau = Nauplii, Cop = Copepodites, Adu = Adult copepods, Hyd = Hydromedusae and Part = Particles of the depicted size range).Effects cannot be assigned to specific covariates in case of correlations between covariates >0.7 (cor).

Phase	Prey category	Covariate	F	p	cor	R^2
II	Zooplankton abundance	CO_2	0.33	0.57	-	0.21
		Nau	6.43	<0.05		
		Ciliates	3.77	0.06		
II	Particle abundance	Hyd	6.09	<0.05	-	0.15
III	Zooplankton abundance	CO2	3.22	0.09	Herring	0.50
		Nau + Cop	19.46	<0.001	-	
		CO_2 * (Nau + Cop)	7.25	<0.05		
III	Particle abundance	Particle 100-600 μm	10.35	<0.01	-	0.40
		Hydromedusae	9.84	<0.01		
IV	Zooplankton abundance	CO2	7.18	<0.05	Herring	0.43
		Cop + Adu	0.13	0.72	-	
		Hyd	16.31	<0.001		
		CO_2 * (Cop + Adu)	1.90	0.18		
IV	Particle abundance	CO2	7.97	<0.01	Herring	0.49
		Part 100-800 μm	15.61	<0.001	-	
		Hyd	6.39	<0.05		

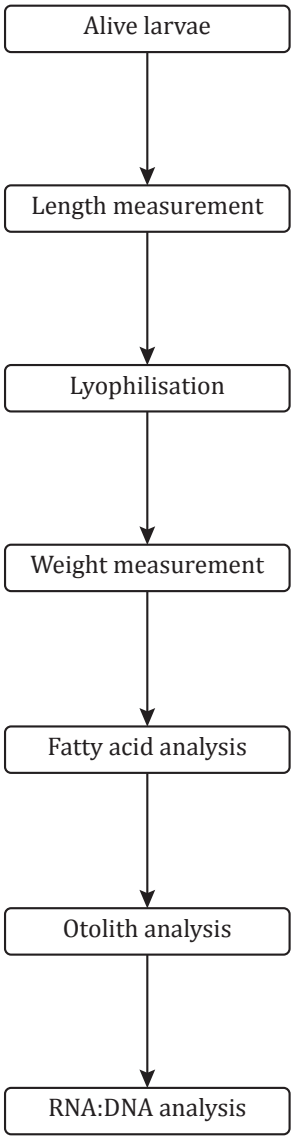

Figure 4.2: Flow chart for stepwise analysis of herring larvae.

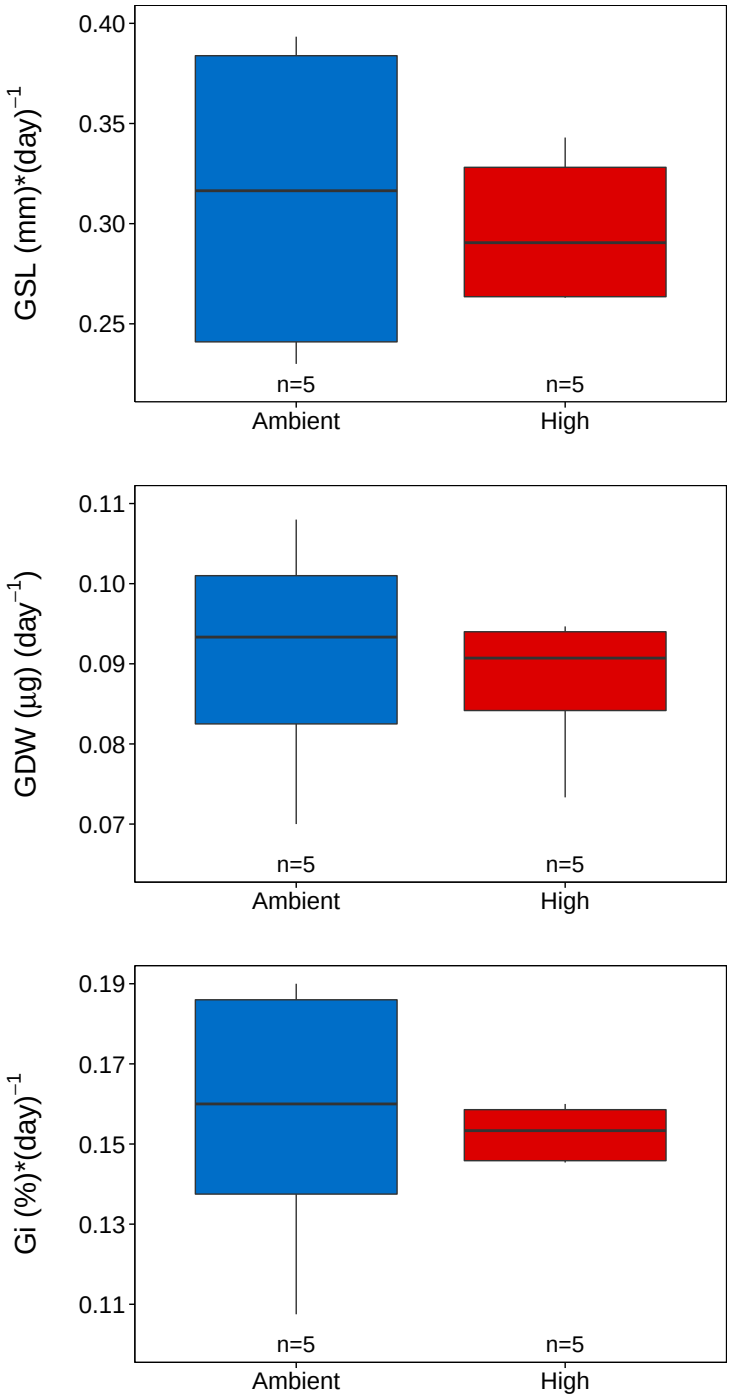

Figure 4.3: Mean growth in a) standard length (G_{SL}), b) dry weight (G_{DW}) and c) instantaneous growth rate (G_i) per CO_2 treatment for the survivors at the end of the experiment (42 DPH) (ambient CO_2=blue, high CO_2=red).

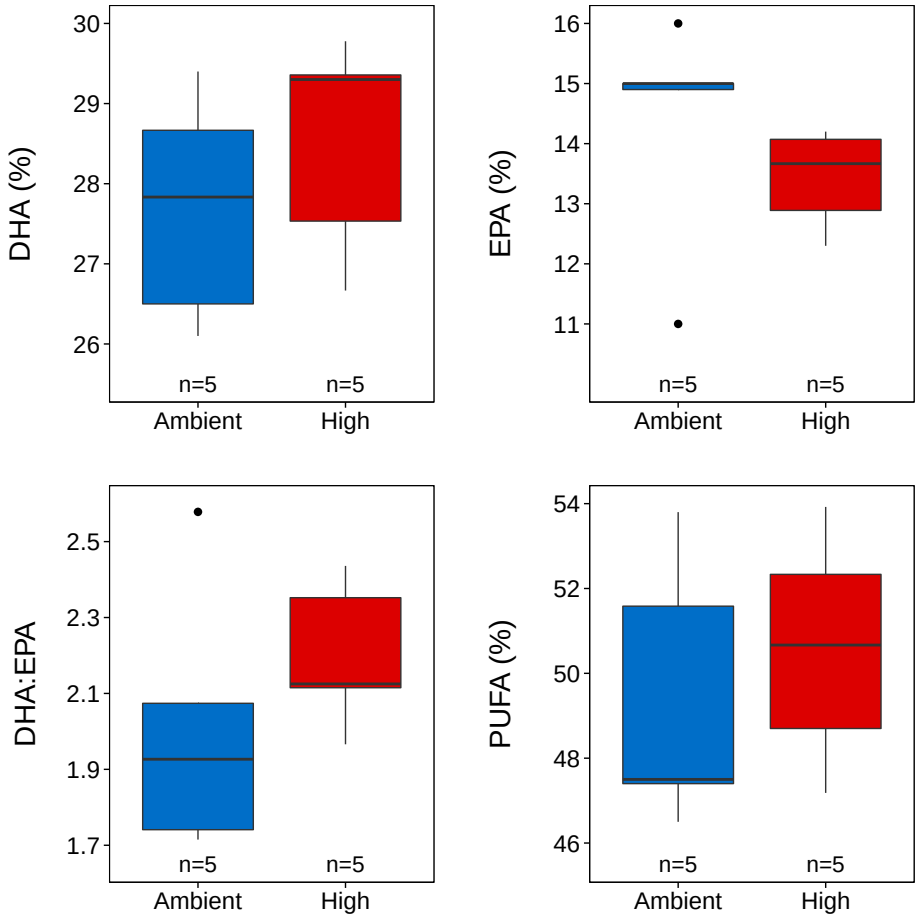

Figure 4.4: Mean content of a) DHA, b) EPA, c) DHA:EPA and d) PUFA in % of total fatty acid content per CO_2 treatment for the survivors at the end of the experiment (42 DPH) (ambient CO_2=blue, high CO_2=red).

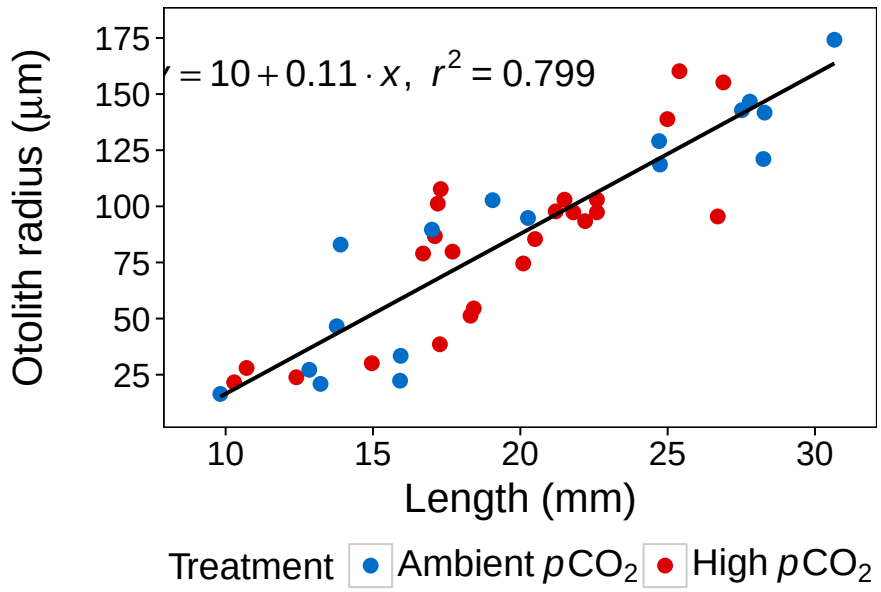

Figure 4.5: Correlation of herring larval length (mm) and radius of the sagittal otolith (μm) with the respective formula and r^2, for both treatments combined (ambient CO_2=blue, high CO_2=red).

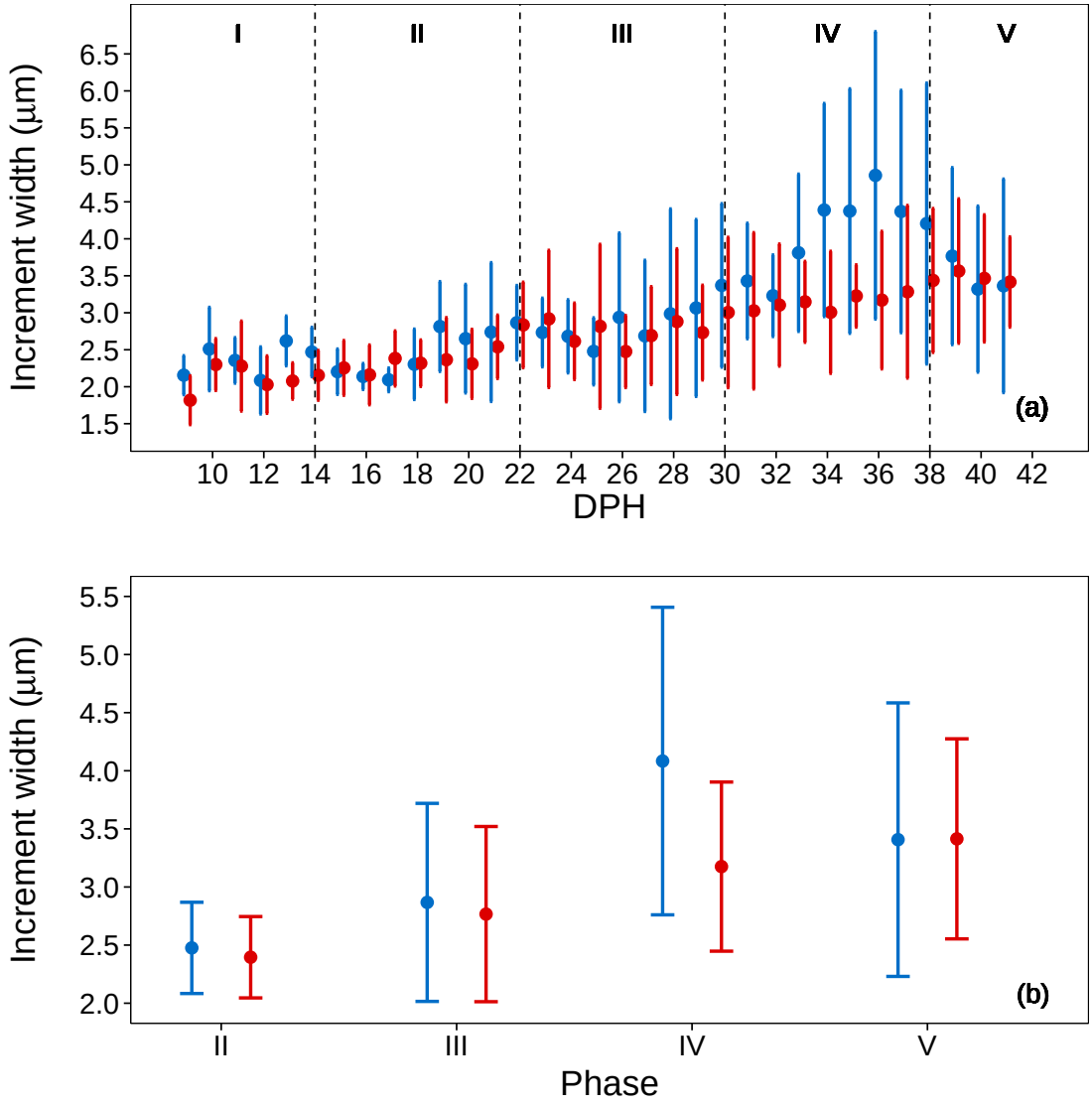

Figure 4.6: Mean otolith increment width (μm) per treatment over time a) in days post hatch (DPH) and b) in the selected phases (ambient CO_2=blue, high CO_2=red).

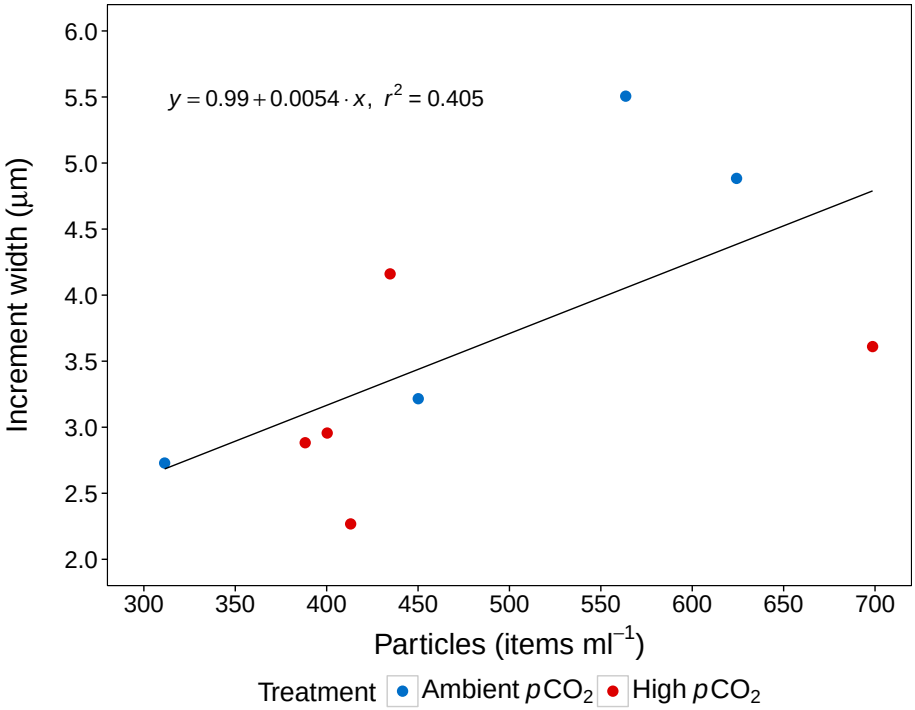

Figure 4.7: Correlation of mean increment width to abundance of particles per ml of the size 100-300μm with the respective formula and r^2, per mesocosm (ambient CO_2=blue, high CO_2=red), in phase IV.

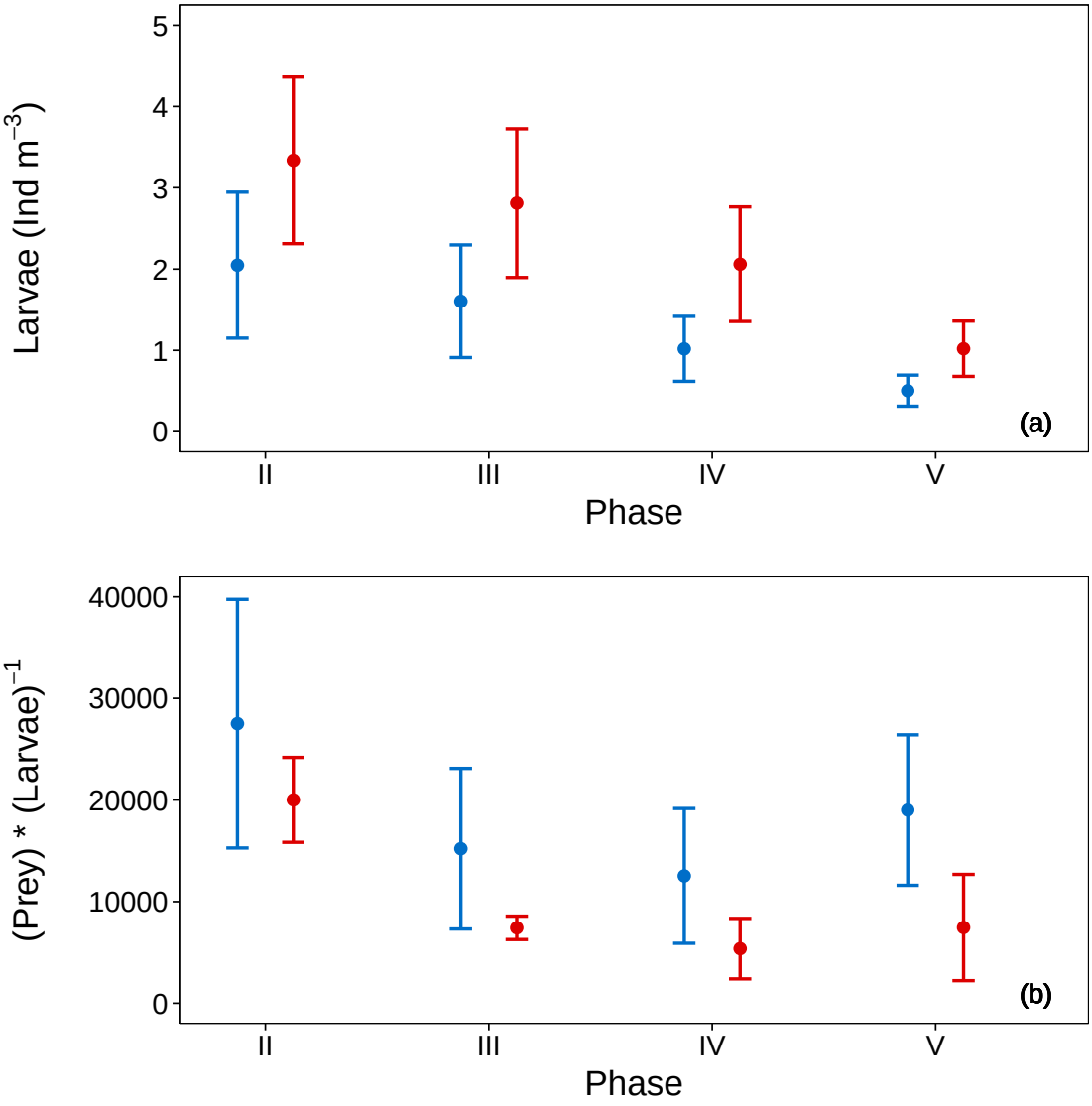

Figure 4.8: Average a) abundance of herring larvae (Individuals m^{-3}) and b) prey availability (items m^{-3}) per herring larvae (Individuals m^{-3}) in the selected phases (ambient CO_2=blue, high CO_2=red). Prey availability is here depicted by the summarized abundances of nauplii and copepodites.

5 | Manuscript IV

Ocean acidification effects on Atlantic cod larval survival and recruitment to the fished population

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RESEARCH ARTICLE

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

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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.

Introduction

The understanding of the effect of global change on fish populations is critical for sustainable exploitation and management of fisheries [1]. Ocean warming has already triggered poleward

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range shifts of many marine fish populations caused by their thermal tolerance [2–4]. 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) [5], is an inevitable consequence of anthropogenic release of CO_2. The potential consequences of ocean acidification on commercially important fish populations are intensely debated [6,7], but currently unresolved since data on population-level processes, e.g. recruitment to the stock, are almost entirely lacking [8–10].

Adult fishes have been shown to tolerate extreme CO_2 concentrations of up to 16,000 μatm [11], which led to the premature conclusion that fishes are less vulnerable to ocean acidification than for example calcifying organisms [12]. 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 [14]. Recent studies have shown a diverse range of impacts of predicted future CO_2 concentrations on larval fish, particularly on sensory abilities like olfaction [15], behaviour [16,17], otoliths [18–20], development, tissue and organ structure [13,21]. Studies also found effects on survival of eggs, more specifically hatching success [22], and survival of very early larval stages [7,23]. Other studies were not able to find an effect on survival [24,25].

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 [26,27] 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 [28]. 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.

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 Table A in S1 File). 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 m3) 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 CO2) or increased CO2 (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 Table B in S1 File). It should be noted, that even though the low food treatment only provided a fraction of the total amount of prey of the high food treatment, it is likely still higher than prey densities, which the larvae would experience in the field. However, this is difficult to compare, since we provided very high densities for short periods at the feeding times, which were then washed out of the tanks again. Therefore no steady density of prey was provided, but during feeding times prey densities were extremely high. This allowed for the exclusion of density and competition effects, which may have otherwise arisen due to different larval densities in the different treatments. Larvae in one tank in the ambient CO2 treatment were abruptly lost over night, due to an unknown factor, resulting in six replicates for the high CO2 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 [31]. 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 were 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 α 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 [20]. 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.

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 (Fig 1a) and from 9.2 to 20.4% in the Western Baltic Sea stock (Fig 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 Tables C and D in S1 File). 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.

Next, the experimentally assessed larval mortality rates were incorporated into a Ricker-type stock-recruitment model that was parametrized for the two studied cod populations. We

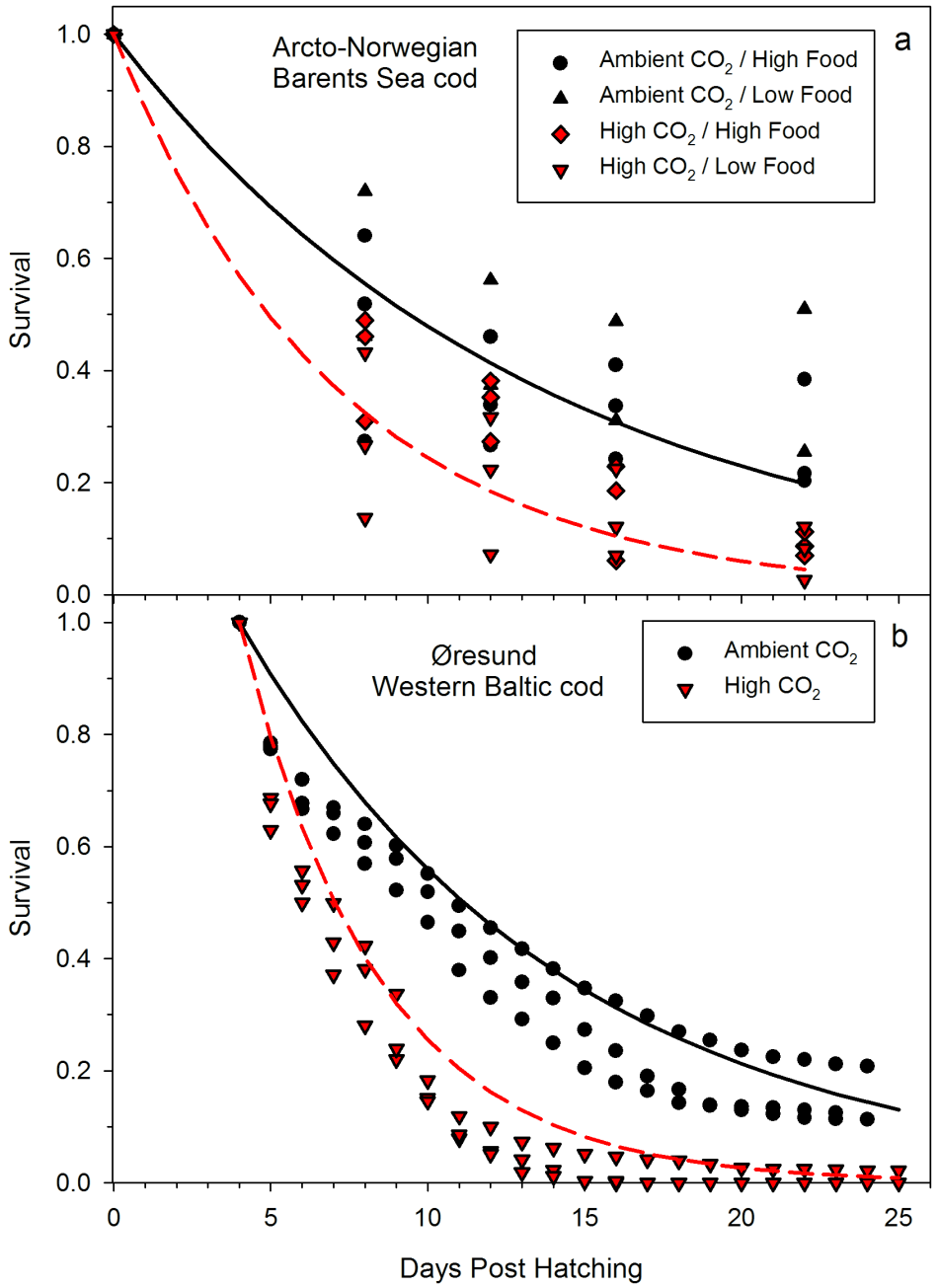

Fig 1. Effect of increased CO_2 on early life survival of *Gadus morhua* from (a) Barents Sea cod (b) Western Baltic cod. Each symbol represents the value of one replicate tank. Lines depict the number of survivors according to the fitted negative exponential function.

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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 (Figs 2 and 3).

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 [27]. Other studies find similar mortality rates as our control values in the two experiments during early larval development [32,33]. 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) [7], albeit in a non-commercial fish species, the Atlantic silverside (*Menidia menidia*). In their study reduced larval survival was observed at 1100 *ppm*, a level of ocean acidification, which is predicted to occur globally at the start of the next century under the IPCC RCP 8.5, during the first week post hatch. Chambers *et al.* (2013) [22] found a decreased hatching success (reflecting embryonic development) of the summer flounder by 50% under 1860 *ppm*. This is 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) [25] found no effect on the survival of yellowtail kingfish larvae. Other studies, like Munday *et al.* (2009b) [24]; Franke & Clemmesen (2011) [34]; Frommel *et al.* (2013) [35]; Hurst *et al.* (2013, 2015) [36,37], 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 [35]. 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 [38]. 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.

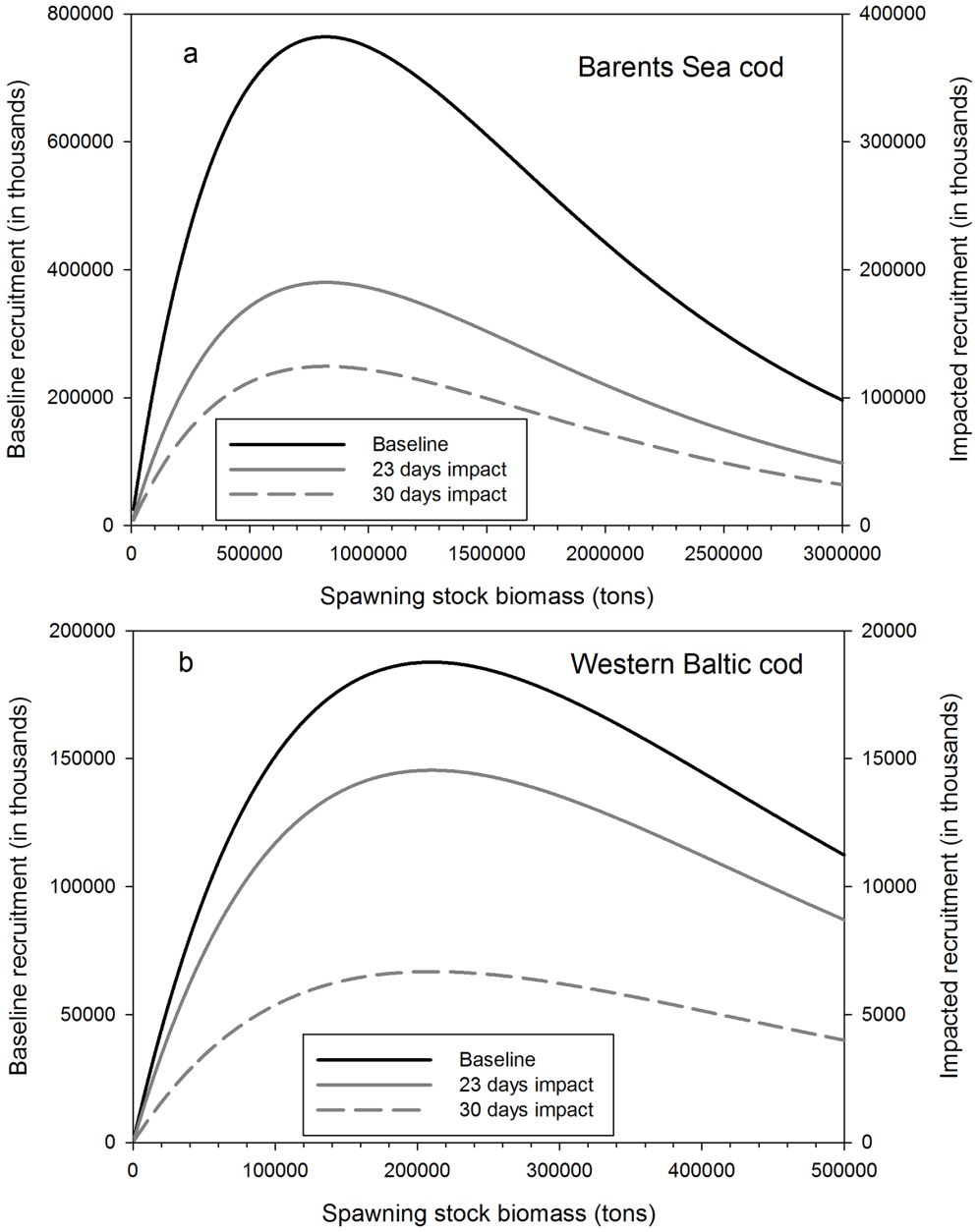

Fig 2. Recruitment functions under baseline and under ocean acidification scenarios for (a) the Barents Sea cod and (b) the Baltic Sea cod. The baseline scenario is based on no OA and spawning stock biomass at ICES precautionary biomass levels (B_{PA}) in dependence of the duration of OA-induced mortality. For better visualization a different scaling on the second y-axes was chosen for the impacted recruitment.

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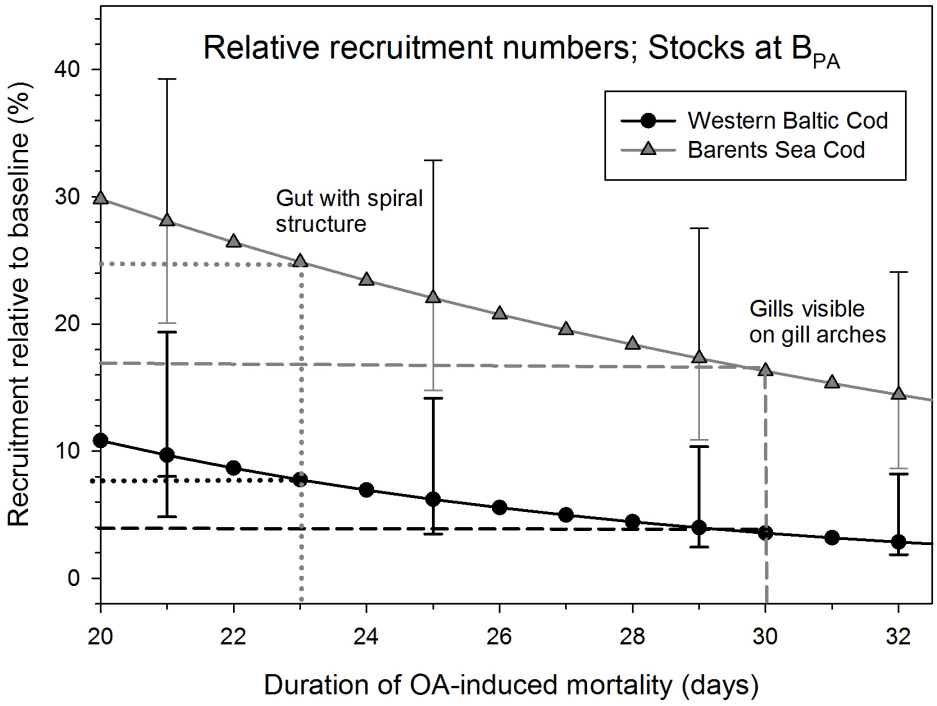

Fig 3. Population recruitment under ocean acidification (OA) for Western Baltic cod (black line and symbols) and Barents Sea cod (grey line and symbols). Recruitment is given relative to a baseline scenario of no OA and spawning stock biomass at ICES precautionary biomass levels (B_{PA}) in dependence 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.

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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 [39]. 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 [2]. 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 [40]. Previously, ocean acidification has been shown to affect marine fish larvae's sensory abilities, morphology of the otoliths, cause tissue damage and behavioural differences [13,17,18,19,21].

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 [41] 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.

Supporting Information

S1 File. Supporting Information on experimental set-up, carbon chemistry, statistics and recruitment modelling.
(DOCX)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: MHS CC MS. Performed the experiments: MHS FHM MS FJ CC VP AM. Analyzed the data: MHS TBHR. Contributed reagents/materials/analysis tools: MC RV. Wrote the paper: MHS TBHR FHM RV CC.

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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 Table A) and with *Nannochloropsis*.

SI Table A. 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 Table B.

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

dph	*Nannochloropsis* added	prey organisms ml^{-1} $feeding^{-1}$	Low Food number of daily feedings	Low Food Prey organisms per day per tank (ml)	High Food number of daily feedings	High Food Prey organisms per day per tank (ml)
1	yes	3.2	7	4.27	7	4.27
2	yes	3.2	7	4.27	7	4.27
3	yes	3.2	7	4.27	7	4.27
4	yes	3.2	7	4.27	7	4.27
5	yes	5.5	7	7.35	7	7.35
6	yes	5.5	7	7.35	7	7.35
7	yes	5.5	7	7.35	7	7.35
8	yes	5.5	7	7.35	7	7.35
9	yes	5.5	7	7.35	7	7.35
10	yes	5.5	7	7.35	7	7.35
11	yes	5.5	7	7.35	7	7.35
12	yes	5.5	7	7.35	7	7.35

13		5.5	7	7.35	7	7.35
14		5.5	3	3.15	7	7.35
15		5.5	3	3.15	7	7.35
16		5.5	3	3.15	7	7.35
17		5.5	3	3.15	7	7.35
18		5.5	3	3.15	7	7.35
19		5.5	3	3.15	7	7.35
20		5.5	3	3.15	7	7.35
21		5.5	3	3.15	7	7.35
22		5.5	3	3.15	7	7.35

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(*1*).

Analytical methods for C_T (total carbon) and A_T (total alkalinity) determination in seawater samples are fully described in *Dickson et al.,* (2007)(*2*). 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(*3*)) to calculate all the other parameters in the CO_2-system such as pH in situ, CO_2 fugacity and partial pressure (fCO_2, pCO_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(*4*) and the CO_2-system dissociation constants (K^*_1 and K^*_2) estimated by Mehrbach et al., 1973(*5*) refit by Dickson and Millero (1987)(*6*). Mean values and standard deviation of pCO_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 pCO_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.

86

87 **Statistics**

88 Data were cubic-root transformed to achieve variance homogeneity, assessed
89 with Bartlett's test. Results are shown in Table C and D.

90 SI Table C. 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

91

92 SI Table D. 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

93

94

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(*7*). 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[8,9].

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[10]. We assume that the stock-recruitment relationship is of the Ricker[11] type. Such a type of stock-recruitment relationship is an appropriate description of recruitment biology of cod[12]. According to the Ricker model[11,13], 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 (5). 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 $\xi_{t+1} = \nu\xi_t + \varepsilon_t$, 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;

132 0.553] and ϕ_2 = 4.762/million tons with 95% confidence interval [-4.672;
133 14.196]/million tons for Western Baltic cod.
134
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168

6 | Synthesis

The aim of my thesis was to test the effect of realistic end of the century CO_2 levels on survival of early life stages of two commercially important fish species. The main focus was on the importance of direct physiological impacts and indirect food web effects of ocean acidification. In the following I will synthesize and discuss the results obtained in chapters (2, 3, 4, 5) and will depict open questions and directions for future research.

6.1 Physiological effects of OA on the early life stages of Atlantic herring and Atlantic cod

Results presented in chapter (2) suggest that herring larvae seem to be mostly tolerant to elevated CO_2 levels in terms of survival, growth in length and weight, growth potential, activity and development within a realistic future ocean acidification range ($\sim$900 μatm pCO_2). These findings are in line with conclusions drawn from a previous study, where herring eggs and newly hatched larvae were mostly unaffected by pCO_2 $\sim$1800 μatm (Franke and Clemmesen, 2011). Other studies showed that behavior and proteome structure of older herring larvae also appear to be unaffected even at pCO_2 levels up to $\sim$4000 μatm (Maneja et al., 2014, 2015). The tolerance of herring larvae to pCO_2 levels $\sim$900 μatm was verified even at low energy supply in the study presented in chapter (2).

In contrast Atlantic cod larvae showed decreased survival at pCO_2 levels $\sim$1000 μatm as presented in chapter (5). This sensitivity to OA of Atlantic cod was also observed for the egg stages. Dahlke et al. (2016) showed that hatching success and size at hatch decrease at pCO_2 levels $\sim$1100 μatm. At very high pCO_2 levels (> 1800 μatm) both herring and cod larvae were shown to suffer from organ damage, which may also induce higher mortality (Frommel et al., 2014, 2011), but it remains hypothetical if this effects can also be observed at pCO_2 levels predicted for the end of the century.

6.2 Differences in life strategy between Atlantic herring and cod

Reasons for the different physiological responses to OA in Atlantic herring and cod may originate from different strategies in the early life history of the two species. Having benthic eggs Atlantic herring embryos experience a variety of physiological stressors and larvae hatch into a naturally variable environment (Geffen, 2009), an extreme example for this can be found in the Baltic Sea and Kiel Fjord, where large fluctuations of CO2 occur commonly (Thomsen et al., 2010; von Dewitz, 2012). In contrast, the larvae of Atlantic cod hatch from pelagic eggs in the surface layers of a fully marine habitat, where pCO_2 levels are reasonablly stable and in equilibrium with the atmosphere. In those regions, pCO_2 levels > 1800 μatm may not be expected in the near future, while they can occur in regions where CO_2 values are naturally high, e.g. areas of upwelling, CO_2 vents and coastal regions (Feely et al., 2010; Cai et al., 2011; Melzner et al., 2012; Hofmann et al., 2011). Thus, early life stages of herring have already been exposed to a more variable environment including higher CO_2 values and may already have adapted to those, whereas Atlantic cod eggs and larvae cannot easily cope with elevated CO_2

levels, which would explain the different effects of future CO_2 levels at the egg and larval stage of both species. Adaption processes may also explain the different effects of OA on the eggs of Atlantic cod from the North Sea and Baltic Sea. Cod eggs of the Baltic Sea populations were shown to be robust to pCO_2 levels as high as $\sim$3200 μatm (Frommel et al., 2012). Compared to Atlantic cod eggs being spawned close to the surface, the eggs of Baltic Sea cod are spawned below the halocline, where CO_2 levels are increased and oxygen is decreased due to higher microbial activity and respiration. Owing to their demersal life style also adult cod in the Baltic Sea live in layers with higher CO_2 levels than their North Sea counter parts.

Environmental stressors often trigger genetic variability, especially if the stressor is of intermittent spatial or temporal frequency (Hoffmann and Hercus, 2000). Recent findings suggest that sensitivity to CO_2 can also be inherited via parental conditioning, as shown for offspring of Atlantic silverside, *Menidia menidia* (Murray et al., 2014). Silverside larvae born from eggs in a naturally low CO_2 season showed a strong decrease in survival when confronted with elevated pCO_2 levels, while those larvae born from eggs in a seasonally high CO_2 environment were unaffected (Murray et al., 2014). In general highly variable pH environments like the Baltic Sea and coastal regions may select for CO_2-tolerant genotypes during early life, while "well-buffered? systems, such as the open ocean, do not (Kelly and Hofmann, 2013). Also trans-generational adaptations may have the potential to reduce detrimental effects of climate change, i.e. a parental acclimatization allows the offspring to cope better with environmental stressors (Donelson et al., 2011). Parental and especially maternal effects can also be related to larval growth and multiple other traits (reviewed in (Green, 2008). As the egg size increase with female size/age also larval size at hatch and growth increases, resulting in better survival and recruitment (e.g. in herring (Blaxter and Hempel, 1963; Høie et al., 1999) and cod: (Kjesbu, 1989; Meekan and Fortier, 1996; Paulsen et al., 2009). This cannot be verified in our studies for herring and cod, as the parental generation was caught in ambient conditions during the spawning season. Thus the big question remains whether CO_2-sensitive species such as Atlantic cod could adapt rapidly, e.g. through trans-generational plasticity, however the life history, such as long generation times and laborious rearing from high larval and juvenile mortality in cod and herring, complicate such experiments.

6.3 Food availability determines OA response in fish larvae

In the study presented in chapter (2), instantaneous growth rates and survival of herring larvae were reduced in the elevated temperature treatment, while CO_2 did not affect these traits. The negative effect of elevated temperature was most likely generated by the combination of non-*ad libitum* (i.e. not sufficient/restricted) feeding conditions and a temperature-induced increase in metabolism. Previous studies reported that marine organism might be able to better cope with abiotic stress (e.g. from OA) by increasing food uptake, if supply is sufficient (Nowicki et al., 2012; Thomsen et al., 2013). However, results from the study presented in chapter (5) suggest that higher food availability did not compensate the negative OA effect on survival in cod larvae from the Barents Sea population. Since in nature feeding conditions are highly variable, the potential of food supply to alter reactions to abiotic stressors, may increase the importance of indirect effects via the food web (Kiørboe et al., 1987). At times of altered prey quantity or quality, species adapted to variable and low feeding conditions may benefit. For example

herring larvae need a lower total zooplankton production to support growth and survival than cod larvae, due to lower metabolic rates (Folkvord et al., 2009; Peck et al., 2012). Differences in critical prey limits of these two species underline this suggestion (Werner and Blaxter, 1980; Puvanendran and Brown, 1999; Folkvord et al., 2009). Furthermore species with a narrow range of prey organisms and thus high selectivity may have a lower potential to substitute altered prey field compositions. In this case herring may also have an advantage over cod, as herring larvae choose from a wider size range of prey items (Peck et al., 2012; Munk, 1997). But when rearing both species in a common garden experiment, Folkvord et al. (2015) found that cod larvae have higher growth rates under medium to high food concentration. Thus in times of a increased prey availabilities, larvae with higher growth rates, e.g. Atlantic cod have an advantage when competing for the same prey source, while slower growing larvae such as Atlantic herring may be better competitors under low food conditions.

The effect of OA on potential prey items for larval fish, like microzooplankton and the different mesoplanktonic copepod stages, seems to be highly variable between species and life stages, but in general these groups are considered tolerant to end of the century CO_2 levels (Isari et al., 2015; Meunier et al., 2016; Cripps et al., 2014; Hildebrandt et al., 2015; Runge et al., 2016). Earlier studies on the indirect effect of OA on higher trophic levels via food web interactions reported negative indirect bottom-up effects of OA from selected phytoplankton to zooplankton species (Rossoll et al., 2012; Cripps et al., 2016). Similarly, bottom-up effects were shown to occur within a plankton community (Bermúdez et al., 2016, 2015), but copepods were always the highest trophic level involved. Hence, only assumptions were made on how these impacts could affect higher trophic levels like fish larvae. Chapters (3, 4) contain results from the first study, where fish larvae were reared together with their naturally occurring prey at end of the century CO_2 concentrations in an in-situ mesocosm approach. Results presented in chapter 3 show, that herring larvae indirectly profited in terms of survival from OA-induced increase in primary production, which fueled an increase in the abundance of prey organisms during the critical first feeding period.

Increasing phytoplankton abundances through higher carbon availability due to OA has been reported for small-sized phytoplankton species (Rost et al., 2008; Low-Décarie et al., 2014; Sala et al., 2015; Crawfurd et al., 2016). Whether or not this phytoplankton biomass fuels secondary production depends on plankton community composition and food web configuration. Direct observations from other studies suggest, that ciliates belong to the prey of first feeding herring larvae (Illing et al., 2015; Bils et al., 2016). In our study, ciliate abundance alone or in addition to nauplii and copepodite abundance did not improve the relation between food abundance and herring larval survival. The prey-predator relationship of both groups could, however, be masked by various factors. Herring larvae may feed preferentially on copepod stages, and thus ciliates could have been released from competition or predation. The latter is indicated by the increase in ciliate abundance after first feeding, which implies a switch to bigger organisms and a top-down release by declining copepod abundances (Horn, subm). As interlinked trophic level, ciliates can also lead to an improvement of food quality in prey organisms of fish larvae via “trophic upgrading? (Klein Breteler et al., 1999) and thus indirectly supporting fish larval performance.

Biochemical composition of prey organisms like fatty acid compositions may affect growth and survival in fish larvae as shown by (St. John, 2001; Cutts et al., 2006; Paulsen et al., 2013a).

Unfortunately, prey quality in terms of C:N ratios or fatty acid composition of zooplankton was not analysed in our mesocosm study. However, the fact that essential fatty acid composition of the surviving herring larvae was unaffected by elevated CO_2 levels, suggests no effect of OA on food quality in the last weeks of the study (Chapter 4). Changes in the C:N ratio of phytoplankton were not significantly affected by CO_2 (Taucher, pers. comm.), which indicates that changes in quality were probably minor. Previous studies have shown that herring larvae feeding on prey (copepods) from a phosphorus-limited food chain had an inferior nutritional condition compared to those reared on a nitrogen-limited or non-nutrient limited food chain (Malzahn et al., 2007). In the mesocosms, herring larvae fed on plankton living under nitrogen-limited conditions and thus potentially no impact on larval nutritional condition. Field studies on herring suggest that prey quantity and quality can similarly affect larval growth, with high prey quantities substituting for low quality prey and vice versa (Paulsen et al., 2013b). This implies that food quality was either not affected by CO_2 in our study or that increased prey abundances compensated for a reduced quality of the prey. Herring larvae in our study may thus have been primarily affected by the changes in prey quantity. Due to the potential interaction of prey quantity and quality, future investigations should cover both aspects of the food web, especially in situation of low prey conditions.

Performance of CO_2-tolerant fish larvae could only be affected indirectly via positive or negative effects of OA on their prey organisms (Fig. 6.1a, c). The question arising from this is, if the direct negative effects of OA in CO_2-sensitive fish larvae could be mitigated or even compensated for by an indirect positive effect of CO_2 via the food web (Fig. 6.1b). Under the scenario of a combined negative direct physiological and indirect food web effect of OA, detrimental impacts observed may even be intensified in CO_2-sensitive fish larvae (Fig. 6.1d). This highlights the need for a better understanding of the relative importance of direct and indirect CO_2 effects on early life stages of CO_2-sensitive species.

6.4 Interaction between competitors under OA

Fish larvae are not only affected by prey availability but also by the occurrence of competitors. Competition between herring larvae most probably prevented a beneficial OA-induced increase in prey abundance on herring larval growth (Chapter 4). Higher herring larval densities in the elevated CO_2 treatment may have exerted an increased predation pressure on prey organisms. In total lower food per individual larvae may thus have limited growth in the elevated CO_2 treatment as indicated by otolith increment width analyses. One can only speculate whether reduced prey availability from stronger herring larvae predation pressure would have not only affected growth but also survival at a later time point. Especially at the end of the study the abundance of the preferred food organism (copepodites) was below optimum prey levels given in Werner and Blaxter (1980), though it may be safe to assume that fish larvae did not rely exclusively on these. Hence, an increased survival of early life stages may seem beneficial for recruitment at first sight, but only if the match scenario prevails. Increased survival and thus predation pressure of one group could also decrease prey availability and thus create a mismatch for competing groups. These competitors could be another cohort of the same species, other fish larvae or another taxa e.g. hydromedusae.

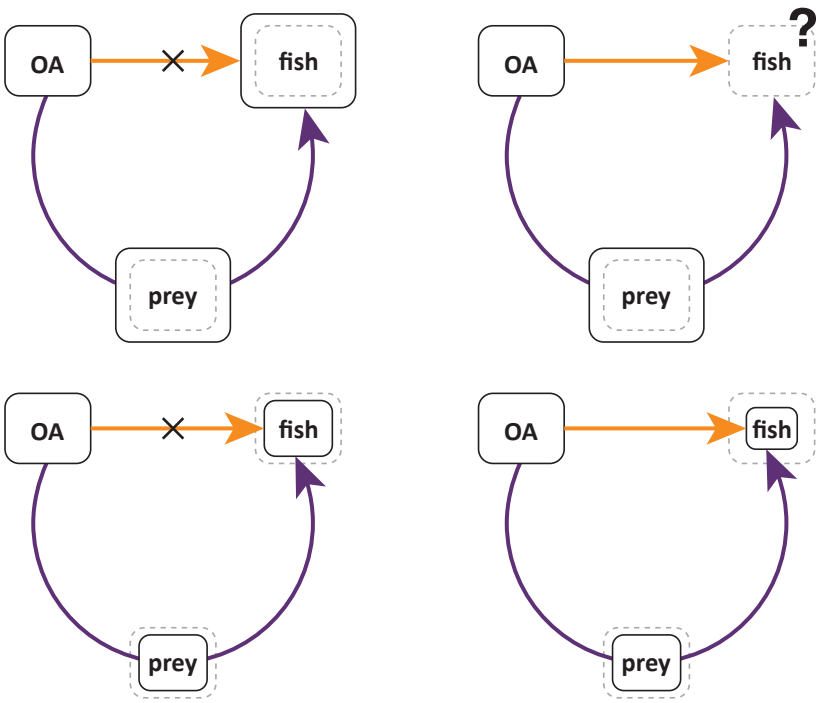

Figure 6.1: Simplified illustration of possible responses of fish larvae to the combined direct physiological (red arrow) and indirect food web (blue arrow) effects of ocean acidification (+, - and x depict a positive, negative and no effect, respectively). The left panel shows the effect of OA on CO_2-tolerant fish larvae under a) positive and c) negative indirect food web effects. The right panel shows the same positive and negative indirect food web effects for CO_2-sensitive fish larvae (b and d, respectively).

6.5 Effect of hydromedusae under OA

Conclusions on the importance of competition between herring larvae and hydromedusae in the mesocosms cannot be drawn easily. If at all, then a positive relation between fish larval growth, estimated from otolith increment width, and hydromedusae abundance was indicated (Chapter 4). Therefore it is possible that prey abundances were sufficient to support both taxa with a low potential for competition, although sharing similar prey organisms (Purcell and Grover, 1990).

Fish larvae will not only have to cope with competition but also with predation by hydromedusae. It is highly unlikely that hydromedusae preyed on herring larvae during our study, based on the size of the hydromedusae (Algueró-Muñiz, pers. comm.) and the interactions described for various species of fish and hydromedusae in the literature (Purcell, 1985). Preliminary results from another mesocosm study on the effect of OA (~2000 μatm pCO_2) on the plankton community of the Raunefjord, Norway, 2015, indicate a negative effect of hydromedusae abundance on herring larval survival (unpubl. data). Similar to the mesocosm study described in chapters (3, 4), herring larval survival was higher under elevated CO_2 levels. Lower survival in the ambient CO_2 treatment was most likely caused by higher predation pressure from gelatinous zooplankton in the first days after hatch combined with lower prey levels. A strong effect of

predation was related to the fact that the majority of herring larvae disappeared during the study.

Theoretically the interaction between species (competition and predation) depends on the OA effect on each rival organism. OA effects can either outbalance each other, if both rivals are affected similarly (Appelhans et al., 2012) or favor one rival over the other (Cripps et al., 2011). While knowledge on the direct effect of OA on fish larvae is accumulating, studies for hydromedusae remain rare. OA was found to decrease the size of statoliths (equilibrium organ) in scyphomedusae (Winans and Purcell, 2010) and may thus also affect hydromedusae statoliths (Purcell et al., 2007). In general hydromedusae are claimed to have comparatively high growth rates and thus a probable advantage over fish (Purcell and Arai, 2001), though this still needs to be assessed more closely with respect to future changes. In general, a broad fundamental knowledge about food web interactions needs to be combined with assessments on physiological effects of CO_2 of each interacting organisms, to unravel potential winners and losers under OA.

6.6 Impact on fish recruitment

As the early life stages of fish are a critical bottleneck for recruitment (Houde, 2008), it is of major importance to detect the potential effects of OA in species, which are already under a strong fishing pressure. Populations of commercially exploited fish species may be affected by the direct impact of overfishing on spawning stock biomass and at the same time by a decreased population size (Swain et al., 2007). The possibility to cope with environmental stressors like OA increases with genetic diversity (Malvezzi et al., 2015). Hence, the lower genetic variability and size/age truncation in fished populations may further decrease the ability to cope with environmental stressors like OA, with consequences for survival and recruitment (Jørgensen et al., 2007; Swain et al., 2007). Both species, Atlantic herring and cod, are of major commercial relevance in the North Atlantic. The far-reaching consequence for cod recruitment originating from a strong direct OA induced decrease in survival was shown in chapter 5. Based on the results from chapter 2, no or only minor direct CO_2 effect on herring recruitment might be expected at pCO_2 levels $\sim$1000 μatm, increasing the importance of indirect food web effects (Fig. 6.1). Differences in recruitment were also related to age/size truncation in several species (review in Hixon et al. (2014)). Older and thus bigger females can spawn more eggs, earlier and over a longer time period than younger ones, as reported e.g. for herring and cod (Lambert, 1987; Hutchings and Myers, 1993; Óskarsson and Taggart, 2006; Marteinsdottir and Begg, 2002). A higher diversity in spawning time and location may buffer recruitment variability from environmental changes (Hixon et al., 2014). A selection for specific phenotypes e.g. by fisheries may thus lead to younger, smaller fish with later spawning times and lower adaption potential to environmental stressors (Hixon et al., 2014), as later spawning times may increase the risk of mismatch between offspring and prey.

6.7 Improvement of mesocosm studies including fish larvae

From a community based point of view changes in the temporal and spatial abundance of interacting species may not only affect population recruitment of some target species, but also key aspects like community composition, trophic transfer efficiency and species coexistence

(Nakazawa and Doi, 2012). Changes in the seasonal overlap between fish larvae, prey and potential competitors/ predators need a closer investigation in the light of ocean warming and acidification. Predation and competition could be included as an additional treatment factor. In mesocosms only temporal aspects of match/mismatch are possible to study, therefore the gained results need to be combined with field observations and model approaches to give a more realistic picture of the spatial match/mismatch effect. Most future ocean processes cannot be tackled in the field, except e.g. in immobile species at natural CO_2 rich sites (Nagelkerken and Connell, 2015). Single processes and underlying mechanisms can be evaluated in lab experiments, but might represent an over-simplification when extrapolated in a spatial and temporal scale to ecological research (Carpenter, 1996). Mesocosm experiments can be used as a link between these two: to test assumptions from the field under more simplified, controlled conditions and to verify results from lab experiments in more realistic conditions. They may also generate new research questions to tackle in small-scale experiments or highlight pathways to improve community and food web models. Mesocosms increase the spatial scale of experiments due to larger volumes incorporated, giving the opportunity to include natural communities with more trophic levels (overview in Riebesell et al. (2013). The duration of the experimental period is elongated due to a decreased potential of interacting artificial factors such as wall growth. Longer durations of experiments increase the possibility that indirect effects via the food web emerge, caused by direct effects on certain groups in the community (Schulz et al., 2013). Although the incorporation of communities provides an opportunity to study effects under close-to-natural conditions, these experiments can reach high complexity, increasing the difficulty to detect effects and interpret results. The importance of effects can still be evaluated, since strong signals may still show up despite higher variability.

Higher trophic levels like fish larvae are difficult to incorporate into mesocosm studies due to the spatial and temporal impact. Due to the strong predation pressure mesocosms incorporating fish larvae need to have sufficient productivity in prey, which is e.g. depending on nutrient availability and standing stock biomass of the plankton. The potential high impact of a top-down control by fish larvae limits the maximum number of fish larvae that can be introduced. This conflicts with naturally high mortalities of fish larvae, requiring for high initial densities of larvae for adequate sample sizes in mesocosm studies. Several studies assessed growth and survival of fish early life stages in mesocosms (Gamble et al., 1985; Øiestad, 1990; Suthers et al., 1999; Folkvord et al., 1994; Vollset et al., 2009) but none of these assessed so far the impact of OA. Some studies achieved high larval densities by artificial re-stocking of prey organisms, which is possible in mesocosms with relatively small experimental volumes (Gamble et al., 1985; Vollset et al., 2009). Other studies used large ponds as mesocosms, with comparatively low densities of fish larvae and thus decreased risk of a top-down control. Due to the big surface are of those ponds horizontal sampling gear for fish larvae could be used, which ensured sufficient sample sizes during the study (e.g. Folkvord et al. (1994)). Small mesocosms with high larval densities were either sampled with water-column-integrated tubes (Vollset et al., 2009) or by simply anaesthetizing the whole water column (Gamble et al., 1985).

The KOSMOS mesocosms (Kiel Off-Shore Mesocosms for Future Ocean Simulations) used for the study presented in chapter (3, 4) enclosed a natural plankton community with no external addition of prey organisms. To minimize the impact of predation by fish larvae, initial numbers were kept relatively low, which in combination with the naturally high mortality lead to relatively

low sample sizes at the end of the study. The diameter of the KOSMOS prevented the use of horizontal sampling methods. Vertical methods like Apstein-nets are considered appropriate sampling methods for homogenously distributed abundant organisms such as copepods. These nets proved ineffective though in catching heterogeneously distributed and mobile organisms like fish larvae during the study, due to their relatively low densities and strong escape behavior. In future KOSMOS studies bigger nets may increase the likelihood to catch sparsely distributed organisms such as fish larvae. The pitfall of bigger nets is the limited number of hauls per mesocosm before an artificial "sampling effect" is observed. The maximum number of hauls is difficult to determine though, as this depends on the "catchability" of other organism in a similar size range as the fish larvae.

For positively phototactic organisms light traps can serve as selective sampling gear and may thus be least influential to other organisms in mesocosms. The efficiency of light traps in catching herring larvae was highly variable in the KOSMOS study. Possible effects could have been density of fish larvae in the vicinity of the light trap, short period of darkness due to geographical location and time and decreased phototactic behavior in later developmental stages of herring larvae.

At the end of the KOSMOS study the remaining larvae, i.e. the survivors, were sampled by a custom-build "ring-net", a foldable net attached to a ring covering the full diameter of the mesocosm. The ring-net is lowered in a folded manner to the sediment trap. By pulling ropes the net unfolds and seals to the ring, entrapping all organisms > 1000 μm above the net i.e. the majority of the mesocosms volume. The net does not fully enclose the sediment trap volume and may thus miss larvae, though this option was minimized by bubbling air through the sediment tube and visual inspection by a camera system. The camera system was also used to get an overall impression at selected days during the study, for example to verify hatch of the larvae. This technique still has to be improved to give reliable counts of living larvae, because they are "rare" and require a large sampling volume, which is a major challenge for current imaging systems. For upcoming KOSMOS studies one solution may be to design a camera-light system, with the visual field covering the full diameter of the mesocosm. Another possibility could be an adaptation of the "*In situ* Underwater Ichthyoplankton Imaging System" (*IS*IIS) described in (Cowen and Guigand, 2008) to fit the mesocosms in handling and size. In situ imaging techniques have big advantages in mesocosms since they are non-invasive and may show rare and fragile organisms, which are not caught in net samples (e.g. (Remsen et al., 2004)). Since the operation of these systems has no impact on the community, number of samples is unlimited and can result in a higher temporal resolution of fish larval abundances and thus survival over time. Still these methods need to be calibrated and tested thoroughly before realistic data can be expected. Concluding on this mesocosms give a unique opportunity to study fish larvae within close-to-natural communities, e.g. under simulated future ocean acidification. For future KOSMOS studies the sampling methods could be improved to expand the knowledge from end-point measurements to several time points. A closer temporal resolution may allow for the assessment of different developmental stages and potential critical phases of fish larvae.

6.8 Perspectives for future research

Differences in the sensitivity of fish larvae to elevated CO_2 levels and the importance of OA-induced changes within plankton communities put the focus of future research on the relative importance of direct physiological and indirect food web effects. It needs to be evaluated how elevated food levels can mediate negative OA effects in CO_2-sensitive species (6.1b). A first step could be to test early life stages of a CO_2-sensitive species such as Atlantic cod at different CO_2 levels and feeding conditions. This way a critical food level may be detected. If food availability is above this level negative direct effects of OA are mediated whereas below this level these impacts can intensify. Performance of CO_2-sensitive fish larvae at different prey levels could thus be compared to empirical data from field, mesocosm and lab studies to detect underlying mechanisms. Both data sets could further be implemented in combined biophysical and individual based models, e.g. for different Atlantic cod stocks by (Hinrichsen et al., 2002; Kristiansen et al., 2014; Pitois and Armstrong, 2014).

In a next step, interaction studies on CO_2-sensitive and -tolerant taxa could detect potential winners and losers of OA. The effect of OA at different food levels could be tested on pairwise comparisons of CO_2-tolerant and CO_2-sensitive groups. These groups could originate from one population (e.g. CO_2-tolerant vs. -sensitive parents), one species (e.g. North Sea vs. Baltic cod), one functional group (e.g. herring vs. cod larvae) or one environment (e.g. hydromedusae vs. fish larvae). The indirect community effect of OA on the selected competitors could be verified in a common garden experiment, where competing groups are introduced together in one treatment combination and individually in other treatment combinations. This way indirect effects of OA via prey availability may be distinguished from indirect competition effects under OA. Finally, the results could serve to build food web models and help to predict potential winners and losers of future ocean acidification. These predictions could be used for capacity building such as adapted management plans for species of ecological and economical importance.

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Eidesstattliche Erklärung

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

Direct and indirect effects of ocean acidification on early life stages of Atlantic cod and herring

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, Dezember 2016 Michael Sswat

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List of publications

Published manuscripts

Stiasny, M. H., Mittermayer, F. H., **Sswat, M.**, Voss, R., Jutfelt, F., Chierici, M., Puvanendran, V., Mortensen, A., Reusch, T. B. H. and Clemmesen, C.: Ocean Acidification Effects on Atlantic Cod Larval Survival and Recruitment to the Fished Population, *PLOS One*, 11, 1-11, doi:10.1371/journal. pone.0155448, 2016

Bach, L. T., Taucher, J., Boxhammer, T., Ludwig, A., The Kristineberg KOSMOS Consortium, Achterberg, E. P., Algueró-Muñiz, M., Anderson, L. G., Bellworthy, J., Büdenbender, J., Czerny, J., Ericson, Y., Esposito, M., Fischer, M., Haunost, M., Hellemann, D.,Horn, H. G., Hornick, T., Meyer, J., **Sswat, M.** , Zark, M. and Riebesell, U.: 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, 13, doi:10.1371/journal.pone.0159068, 2016

Paul, A. J., Bach, L. T., Schulz, K.-G., Boxhammer, T., Czerny, J., Achterberg, E. P., Hellemann, D., Trense, Y., Nausch, M., **Sswat, M.** and Riebesell, U.: Effect of elevated CO_2 on organic matter pools and fluxes in a summer Baltic Sea plankton community, *Biogeosciences*, 12, 6181–6203, doi:10.5194/bg-12-6181-2015, 2015

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Manuscripts under review

Sswat, M., Stiasny, M. H., Jutfelt, F., Riebesell, U. and Clemmesen, C.: Growth performance and survival of larval Atlantic herring, under the combined effects of elevated temperatures and CO_2,*under review, PLOS One*

Manuscripts in preparation

Sswat, M., Stiasny, M. H., Algueró-Muñiz, M., Bach, L. T., Jutfelt, F., Taucher, J., Riebesell, U. and Clemmesen, C.: Indirect food web effects of ocean acidification increase herring larval survival

Sswat, M., Stiasny, M. H., Algueró-Muñiz, M., Bach, L. T., Horn, H. G., Jutfelt, F., Taucher, J., Clemmesen, C. and Riebesell, U.: Effect of ocean acidification on prey availability and growth performance of herring larvae in large scale mesocosms

Schulz, K. G., Bach, L. T., Bellerby, R. G. J., Bermdez, R., Büdenbender, J., Boxhammer, T., Czerny, J., Engel, A., Ludwig, A., Meyerhöfer, M., Larsen, A., Paul, A.,**Sswat, M.** and Riebesell, U..: Phytoplankton blooms at increasing levels of atmospheric carbon dioxide: experimental evidence for negative effects on prymnesiophytes and positive on small picoeukaryotes

Non-peer reviewed publications

Sswat, M., Boxhammer, T., Jutfelt, F., Clemmesen, C. and Riebesell, U.: Performance of herring larvae in a simulated future ocean food web, using the "Kiel Off-Shore Mesocosms for future Ocean Simulations", doi:10.3289/KOSMOS_HERRING_SWEDEN_2013, 2016

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Boxhammer, T., **Sswat, M.**, Paul, A. J., Nicolai, M. and Riebesell, U.: Video of a plankton community enclosed in a "Kiel Off-Shore Mesocosm for future Ocean Simulations"(KOSMOS) during a study in Tvärminne Storfjärden (Finland) 2012, doi:10.3289/KOSMOS_PLANKTON_FINLAND_2012, 2015

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