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Effect of ocean acidification on early life stages of Atlantic herring (*Clupea harengus* L.)

A. Franke and C. Clemmesen

Marine Ecology, Leibniz-Institute of Marine Sciences (IFM-GEOMAR), Düsternbrooker Weg 20, Kiel 24105, Germany

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Correspondence to: A. Franke (afranke@ifm-geomar.de)

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Abstract

Due to atmospheric accumulation of anthropogenic CO_2 the partial pressure of carbon dioxide (pCO_2) in surface seawater increases and the pH decreases. This process known as ocean acidification might have severe effects on marine organisms and ecosystems. The present study addresses the effect of ocean acidification on the early developmental stages, the most sensitive stages in the life history, of the Atlantic herring (*Clupea harengus* L.). Eggs of the Atlantic herring were fertilized and incubated in artificially acidified seawater (pCO_2 1260, 1859, 2626, 2903, 4635 µatm) and a control treatment (pCO_2 480 µatm) until the main hatch of herring larvae occurred. The development of the embryos was monitored daily and newly hatched larvae were sampled to analyze their morphometrics, and their condition by measuring the RNA/DNA ratios. Elevated pCO_2 neither affected the embryogenesis nor the hatch rate. Furthermore the results showed no linear relationship between pCO_2 and total length, dry weight, yolk sac area and otolith area of the newly hatched larvae. For pCO_2 and RNA/DNA ratio, however, a significant negative linear relationship was found. The RNA concentration at hatching was reduced at higher pCO_2 levels, which consequently should lead to a decreased protein biosynthesis. The results indicate that an increased pCO_2 can affect the metabolism of herring embryos negatively. Accordingly, further somatic growth of the larvae could be reduced. This can have consequences for the larval fish, since smaller and slow growing individuals have a lower survival potential due to lower feeding success and increased predation mortality. The regulatory mechanisms necessary to compensate for effects of hypercapnia could therefore lead to lower larval survival and could affect the ecosystem and fisheries. Since the recruitment of fish seems to be determined during the early life stages, future research on the factors influencing these stages are of great importance in fisheries science.

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1 Introduction

The atmospheric CO_2 concentration is constantly increasing primarily due to human activities causing an acidification of the ocean (Feely et al., 2004). While the CO_2 concentration over the last 650 000 years ranged between 180 and 300 ppm the recent global mean is ~391 ppm (Conway and Tans, 2011) and a further rise up to 450 respectively 1100 ppm by the end of the century, depending on the emission scenario, is predicted (IPCC, 2007). As a result the seawater carbonate chemistry is changing and the present mean oceanic surface pH of 7.9–8.25 is expected to decrease by ~0.3–0.5 units (Caldeira and Wickett, 2005). However, there are naturally CO_2 enriched habitats such as upwelling regions (Feely et al., 2008). In the Baltic Sea acidification of coastal surface waters occurs as a result of its strong seasonal stratification, which is causing hypoxia in deeper water layers and subsequent upwelling of CO_2 enriched waters (Thomsen et al., 2010). In our study area, the Kiel Fjord, the pCO_2 is elevated for large parts of the year with peak values of > 2300 µatm during late summer, which could increase to > 4000 µatm in the future according to simple model calculations (Thomsen et al., 2010).

Studies reporting the potential impact of ocean acidification on marine organism used to focus on calcifying organisms (Langdon, 2002; Riebesell, 2004; Shirayama and Thornton, 2005; Berge et al., 2006; Gazeau et al., 2007; Fabry, 2008). Furthermore, a variety of physiological traits such as acid-base regulation, metabolic rate and growth under elevated CO_2 concentrations have been analysed (Larsen et al., 1997; Michaelidis et al., 2007; Metzger, 2007; Melzner et al., 2009a; Gutowska et al., 2010) and reviewed (Ishimatsu et al., 2008; Poertner et al., 2004; Poertner and Peck, 2010). By using meta-analytic techniques it has been shown that the biological effects of ocean acidification are negative yet variable amongst organisms (Kroeker et al., 2010). It is hypothesised that the response of marine organisms to acidified seawater does not only vary between different taxa, but also at the species level (Ries et al., 2009). Generally, organisms with efficient acid-base regulatory mechanisms e.g. fish

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and cephalopods are found to be less adversely affected. However, early life history stages even of the more tolerant taxa are assumed to be most susceptible (Poertner et al., 2005; Melzner et al., 2009b). Considering that early life stages are generally known to be most affected by abiotic conditions such as oxygen availability, temperature and salinity (Blaxter, 1956; Rosenthal and Alderdice, 1976; Bonk, 2005) particular importance should be given to the potential effect of acidified seawater on their development. Unfortunately, only few studies on the influence of ocean acidification on early life stages have been conducted, most of them focussing on invertebrates such as molluscs, crustaceans and echinoderms (e.g. Kurihara et al., 2004; Havenhand et al., 2008; Ellis et al., 2009; Dupont et al., 2010), indicating that the impact of ocean acidification on early life history of invertebrates is highly variable amongst different species, even within closely related taxa (Dupont and Thorndyke, 2009).

So far only very few studies on the effect of hypercapnia on early developmental stages of marine teleosts, using pCO_2 concentrations in the range of future predictions, have been published (Checkley et al., 2009; Munday et al., 2009, 2011a, b). Higher pCO_2 levels (up to 150 000 µatm) were used by Kikkawa et al. (2003) to investigate the acute lethal effect of pCO_2 on early life stages of marine fishes.

In this study we examined to what extent elevated pCO_2 concentrations affect the embryonic development and the condition of newly hatched larvae of the Atlantic herring, a teleost fish of major commercial importance in the Baltic Sea. We tested whether acidified conditions influence the embryogenesis and hatch rate as well as morphometrics, otolith area and RNA/DNA ratio of newly hatched herring larvae.

2 Material and methods

2.1 Experimental setup and water chemistry

Adult Atlantic herring from a local spring-spawning stock were caught in the Kiel Fjord, one of the most important spawning grounds in the Western Baltic Sea (Kafemann et

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al., 1998), in April 2007. The gametes of 3 females and 3 males with a total length of 28 cm each were used to perform a laboratory experiment in filtered (0.5 µm) and UV sterilized seawater from the Kiel Fjord (salinity 14.0) in a temperature constant room set at 12 °C with a day/night cycle of 12/12 h.

We used 10 l gas-proof high-density polyethylene (HDPE) containers firmly closed with lids as experimental units for egg incubation. A centrifugal pump (1005 21–5 Eheim) was attached to every container with one tube each plugged in the aspiration port and the discharge port going through the container lid ensuring a constant water circulation within the sealed systems to avoid fungal infestation on the eggs. Temperature, oxygen content and pH_F (free pH scale) were measured daily in each experimental unit (WTW Multi 350i with SenTix 21 electrode).

We set up 6 different treatment levels (in 4 replicates) composed of a control treatment (untreated Baltic Sea water) and 5 treatment levels with elevated pCO_2 concentrations. The different concentrations were adjusted through addition of a strong acid (1M HCl), according to the Guide to best practices for ocean acidification research and data reporting (Riebesell et al., 2010) one of the most useful techniques to manipulate the seawater chemistry.

Before starting the experiment total dissolved inorganic carbon (C_T), total alkalinity (A_T), temperature and salinity of the stock seawater were determined. C_T was measured photometrically in duplicate after Stoll et al. (2001) using a Bran & Lübbe Quattro Analyzer equipped with a XY-2 autosampler. A_T was measured in duplicate through potentiometric titration after Dickson (1981) with a Metrohm Titrando 808. To quantify the measurement accuracy of C_T and A_T certified reference material (provided by A. G. Dickson, Scripps Institution of Oceanography) was used. Based on the measured C_T and the aimed pCO_2 the resulting A_T of the respective treatment level was calculated with the CO2SYS macro for low salinities (modified by Körtzinger after Pierrot et al., 2006) using the dissociation constants K_1 and K_2 according to Roy et al. (1993) and adjusted by adding the corresponding amounts of 1 M HCl to the stock seawater. At the beginning, intermediate phase and end of the experiment water samples

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for C_T and A_T measurements were taken in each experimental unit and processed as described above.

To reduce the chance of low quality gametes and to simulate natural variability, we incubated eggs from all 3 females in each experimental unit. 50 eggs of each female were strip-spawned on a plastic plate (48 plates in total, each 9 cm × 2.5 cm). The eggs of every female were arranged in a single row to ensure equal gas exchange and comparable environmental conditions. Fertilization was performed in water of the respective treatment level adding a sperm mixture of 3 males. Subsequently, 2 plates each (plate 1 and plate 2) were put in a holder at the bottom of every HDPE container. Fertilization rates were determined 2 h later for every single plate under a stereomicroscope (Leica MZ8). From the second day on eggs of plate 1 were photographed daily with a Canon Digital Ixus camera connected via C-mount to a stereomicroscope (Leica MZ8) to monitor the embryonic development, to determine the proportion of malformed eggs and the overall egg mortality. Plate 2 was not taken out of the containers at any time during the course of the experiment.

To reduce the drift from the originally set pCO_2 levels due to respiration of the eggs, 40 % of the water was exchanged at day 6 in every experimental unit by using stock seawater (untreated and adjusted to the different CO_2 concentrations, respectively) which was stored in completely filled and sealed plastic containers at 12 °C since the beginning of the experiment.

2.2 Analysis of eggs and larvae

After the main hatch occurred, yolk sac larvae were transferred into 1.5 ml Eppendorf safe-lock tubes with seawater and frozen at −70 °C. Hatch rate was determined by counting empty eggshells under a stereomicroscope (Leica MZ8). For the following analysis, larvae were thawed and photographed with a QImaging MicroPublisher 3.3 RTV camera connected via C-mount to a stereomicroscope (Leica MZ95) in order to measure the total length and the yolk sac area using the program UTHSCSA Image Tool 3.0.

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To determine the dry weight, larvae were rinsed in distilled water to avoid salt residues, put individually in 1.5 ml Eppendorf safe-lock tubes and freeze-dried (Christ Alpha 1–4 freeze-drier) for 16 h at −55 °C. They were subsequently weighed to the nearest 0.1 µg (Sartorius microbalance SC2) and either used for removal of otoliths or biochemical analysis.

For otolith removal, larvae were put in a drop of distilled water on a microscope slide. Right and left sagittae and lapilli were quickly (2–3 min) dissected under a stereomicroscope (Leica MS5) equipped with a polarizing filter using 2 fine dissecting needles and fixed with clear nail polish. Digital pictures of the otoliths were taken at 1250x magnification using a microscope (Leitz Laborlux S) equipped with a QImaging MicroPublisher 3.3 RTV camera. Sagitta and lapillus areas were measured with the image analysis software Image-Pro Plus 5.0.

Larvae were analysed for RNA and DNA concentrations using a modification of the method of Clemmesen (1993) and Belchier et al. (2004) as described in Malzahn et al. (2007). For the determination of RNA/DNA ratios, nucleic acids were quantified fluorometrically in a microtitre fluorescence reader (Labsystems, Fluoroskan Ascent) using ethidium bromide as a fluorophore. For RNA and DNA calibrations 16S and 23S rRNA (Boehringer 206938) and Lambda DNA (Boehringer 745782), respectively, were used. RNA amounts were calculated using the RNA standard calibration curves. DNA amounts were calculated using the relationship between RNA and DNA fluorescence described by Le Pecq and Paoletti (1966) resulting in a slope ratio of 2.2 (Caldarone et al., 2006).

2.3 Statistical analysis

Since the experiment was set up to evaluate a broad range of pCO_2 levels, linear regression analysis was the statistical method of choice. Statistical analyses were performed using the software Statistica 6.1 (StatSoft, Inc.). All data were tested for normality using the Shapiro-Wilk test. Non-normally distributed data were log transformed and percentage data were arcsine transformed prior to linear regression analysis. Data

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were tested for homogeneity of variances using Levene's test if a significant linear relationship was found. The difference between right and left otolith areas (sagitta and lapillus, respectively) was analyzed using a paired t-test after data were tested for normality and homogeneity of variances. Since no difference between right and left otolith areas was observed, the data were combined and the resulting mean values were used for linear regression analysis. We also calculated effect sizes and 95 % confidence intervals around effect sizes using the results from control (480 µatm) and highest treatment (4635 µatm) applying the methodology of Hedges and Olkin (1985).

3 Results

We incubated herring eggs at 6 mean pCO_2 values of 480 ± 81, 1260 ± 218, 1859 ± 240, 2626 ± 197, 2903 ± 204 and 4635 ± 340 µatm (corresponding to pH_F values between 8.08 ± 0.07 and 7.05 ± 0.03) until the main hatch occurred (Table 1). Due to storage problems the C_T water samples could not be used, thus the carbonate system was calculated using measured pH_F and A_T values.

The mean oxygen content was above 7.2 mg l^{-1} in all cases until the end of the experiment. The incubation temperature (mean ± SD: 13.6 ± 0.4 °C) was above the set room temperature (12 °C) due to the heat production of the pumps attached to the experimental units.

Fertilization was successful, resulting in rates between 86 and 90 % at all treatment levels. Neither the daily observation of the herring eggs nor the evaluation of the daily taken digital photographs showed any difference or time delay in the embryonic development between the 6 treatment levels. The herring embryos showed the same stage of development regarding blastoderm formation, epiboly, appearance of eyes and myomeres, beginning of embryonic movement, heart pulsation, eye pigmentation, appearance of otoliths and main hatch at the respective time of monitoring.

There was neither a significant linear relationship between the pCO_2 level and the incidence of embryonic malformations such as deformation and irregular cleavage of

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blastomeres (Fig. 1a; $r^2 = 0.02$, $P = 0.52$), nor the mortality rate during the embryonic development (Fig. 1b; $r^2 = 0.02$, $P = 0.53$).

There was no effect on the embryonic duration, since the main hatch occurred at the night of day 8 at all pCO_2 conditions. The hatch rate varied between 66 and 96 %, except for one replicate of the highest treatment level having a hatch rate of only 48 %. However, no significant linear relationship between pCO_2 level and hatch rate was found (Fig. 2; $r^2 = 0.09$, $P = 0.17$).

The elevated pCO_2 conditions neither affected the total length ranging from 5.91 to 6.96 mm (Fig. 3a; $r^2 = 0.001$, $P = 0.87$), the dry weight ranging from 38.8 to 54.3 µg (Fig. 3b; $r^2 = 0.07$, $P = 0.23$), nor the yolk sac area ranging from 0.38 to 0.60 mm^2 (Fig. 3c; $r^2 = 0.03$, $P = 0.40$) of the newly hatched larvae.

The left and right otolith areas did not differ significantly from each other (paired t-test for sagitta and lapillus, respectively: $P > 0.05$). The mean sagitta area varied from 371 to 470 μm^2 and the mean lapillus area from 314 to 419 μm^2. No significant linear relationship between the pCO_2 level and the otolith area was found (sagitta: Fig. 4a; $r^2 = 0.02$, $P = 0.47$; lapillus: Fig. 4b; $r^2 = 0.10$, $P = 0.13$).

In contrast to all the other examined parameters, the RNA/DNA ratio, ranging from 2.5 to 3.8, was negatively affected by acidification (Fig. 5a; $r^2 = 0.47$, $P < 0.01$, y = 3.42–0.00019*x) and the relative RNA content (RNA/dry weight) was lowered significantly ($r^2 = 0.31$, $P < 0.05$, y = 37.72–0.0019*x). However, a significant correlation could no longer be detected when excluding the highest treatment level from the statistical analysis (Fig. 5b; $r^2 = 0.25$, $P = 0.10$).

Calculated effect sizes and 95 % confidence intervals around effect sizes for embryonic malformations, mortality rate during embryogenesis, hatch rate, total length, dry weight, yolk sac area, sagitta area, lapillus area and RNA/DNA ratio based on differences of control (480 µatm) versus highest treatment (4635 µatm) are presented in Fig. 6. These showed clear overlap with zero for all variables tested, except for the lapillus area and the RNA/DNA ratio.

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4 Discussion

4.1 Effects on early development

An emerging body of evidence suggests that the impact of future ocean acidification on marine organisms will be more variable than previously thought, generating winners and losers (Doney et al., 2009). While most studies focus on the effect of rising pCO_2 on marine calcifiers (e.g. Fabry, 2008; Wood et al., 2008; Gooding et al., 2009; Lischka et al., 2011; Appelhans et al., 2011), few studies have examined the potential impact on non-calcifiers (Ishimatsu et al., 2008; Melzner et al., 2009b; Gutowska et al., 2010; Hu et al., 2011). And even though it is widely accepted that early life history stages may be the most sensitive to CO_2-induced ocean acidification (Raven et al., 2005; Poertner and Farrell, 2008), rather limited information about the effect of hypercapnia on early life stages of fishes is available (Kikkawa et al., 2003; Checkley et al., 2009; Munday et al., 2009, 2011a,b).

In this study we examined the effect of ocean acidification on the embryogenesis and the condition of newly hatched larvae of the Atlantic herring, *Clupea harengus*. We found no significant effect of elevated pCO_2 on the occurence of embryonic malformations, the mortality rate of eggs, the embryonic duration, the hatch rate as well as the total length, dry weight, yolk sac area and otolith area at hatching based on linear regression analysis. The only parameters resulting in a significant linear relationship were the RNA content and the RNA/DNA ratio, which showed a decrease with increasing pCO_2. Since non-significant results are inconclusive (Fisher, 1935; Nakagawa and Foster, 2004), additional statistical support can be provided by 95 % confidence intervals around statistical effect sizes (Nakagawa and Foster, 2004). When calculating effect sizes and 95 % confidence intervals around effect sizes for embryonic malformation, egg mortality rate, hatch rate, total length, dry weight, yolk sac area and sagitta area at hatching a clear overlap with zero was found. Therefore, we concluded that the egg stage of *C. harengus* is tolerant to pCO_2 levels up to 4635 µatm, exceeding future predictions of ~4300 µatm for the Kiel Fjord (Thomsen et al., 2010). However, when

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using the effect size statistics a positive effect of ocean acidification on the lapillus area and a negative effect on the RNA/DNA ratio was shown confirming the results from the linear regression analysis for this biochemical indicator.

Our results coincide with the data presented by Munday et al. (2009) who found no detectable effect on the embryonic duration, egg survival, hatch rate and size at hatching of the coral reef fish *Amphiprion percula* at pCO_2 concentrations up to 1030 µatm. *A. percula* is a benthic spawner that lays clutches of eggs on hard surfaces in coral reefs where water pH varies during the day and sometimes reaches values below 8.0. Consequently, the eggs might be adapted to variations in ambient pCO_2 levels (Munday et al., 2009). Herring spawns its benthic eggs on plant substrate or hard substrate during spring, when the pCO_2 according to Thomsen et al. (2010) reaches its minimum (385 µatm) in the Kiel Fjord. However, the pCO_2 of the Kiel Fjord surface water rises from spring to late summer up to a value of ~2300 µatm (Thomsen et al., 2010), thus herring larvae develop under constantly rising pCO_2 conditions.

Gutowska and Melzner (2009) showed that the pO_2 and pH decreases during the embryonic development in cephalopod (*Sepia officinalis*) eggs, while the pCO_2 increases reaching tenfold higher values than those of ambient sea water. Accordingly, pH values of the perivitelline fluid descended down to 7.2. A decrease of pO_2 during the embryogenesis of shark (*Scyliorhinus canicula*) eggs was shown by Diez and Davenport (1987). Since the egg case serves as a diffusion barrier, high pCO_2 values in developing fish eggs are expected and powerful net proton excretion mechanisms should be present already in these early developmental stages to cope with high perivitelline fluid pCO_2 (Melzner et al., 2009b), but this has not been demonstrated for herring eggs yet.

When analysing four different teleost species Kikkawa et al. (2003) found the cleavage and juvenile stages to be the most susceptible to acute CO_2 stress and the most tolerant stages were the embryo, preflexion and flexion stages. The reason for the ontogenetic changes in CO_2 tolerance might be the development of ion-regulatory chloride cells during the course of embryogenesis (Ishimatsu et al., 2004). While cleavage

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stages have no ion-regulatory chloride cells (Katoh et al., 2000), they have been found in the yolk sac membrane and body skin of embryos and larvae in various teleost species (Shiraishi et al., 1997; Hiroi et al., 1998; Sasai et al., 1998; Katoh et al., 2000). Preliminary results from experiments in our laboratory (Bodenstein and Clemmesen, 2011) indicate that these chloride cells are also found in herring embryos. The gradual fall in CO_2 tolerance from larval to juvenile stage observed by Kikkawa et al. (2003) was also shown in Atlantic cod (*Gadus morhua*) by Frommel et al. (2011) and may result from the energy demanding transition from one acid-base regulatory site (yolk sac) to the other (gill) (Melzner et al., 2009b).

4.2 Biochemical indicator – RNA/DNA ratio

Analyses of larval fish nucleic acid ratios provide a powerful tool to analyze and assess larval growth and condition (Clemmesen, 1994; Buckley et al., 1999, 2008; Pepin et al., 1999; Caldarone et al., 2006). The applicability of the nucleic acid ratio is based on the fact that DNA concentrations within individual cells remain fairly constant while RNA concentrations increase as protein synthesis increases (Buckley at al., 1999). The RNA/DNA ratio is therefore used as an indicator of protein biosynthesis and has been shown to be dependent on the nutritional condition and correlated to growth rate (Bergeron, 1997; Clemmesen et al., 1997; Gronkjaer et al., 1997; Voss et al., 2006; Malzahn et al., 2007; Huwer et al., 2011). Hence, the use of the RNA/DNA ratio allows for the determination of sublethal stressors already on the biochemical level, before a change in somatic growth or mortality is observed (Sprague, 1971).

The RNA/DNA ratios of the newly hatched herring larvae were negatively affected by the pCO_2 level. Since the DNA content per larval dry weight did not change in relation to the treatment levels, the number of cells per unit body weight was not affected. The change in the ratio was achieved by a reduction in the amount of RNA, indicating a reduction in protein biosynthesis and machinery. So far a reduction in growth and changes in the metabolic profile under hypercapnia have been shown in juvenile respectively adult fish by Foss et al. (2003) in the Spotted wolffish (*Anarhichas minor*)

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and by Michaelidis et al. (2007) in the Gilthead seabream (*Sparus aurata*). Since the negative linear correlation could no longer be detected when the highest treatment level was deleted, the questions about a potential tipping point cannot be satisfactory addressed from this study.

Even though no effects on size and dry weight of newly hatched herring larvae were observed in this study, the question remains, whether effects could appear later during the larval phase. Results on the impact of ocean acidification on Atlantic cod larvae from mesocosm experiments indicate that the stressor gradually shows an effect on the developing larvae and causes organ damage during transition phases (Frommel et al., 2011).

A reduction in growth as a result of a decrease in protein biosynthesis can have enormous consequences for larval fish, since the smaller and slower growing individuals have a lower survival potential due to lower feeding success and increased predation mortality (Houde, 1987, 2008; Anderson, 1988; McGurk, 1993; Leggett and DeBlois, 1994). Poertner et al. (2004, 2005) and Denman et al. (2011) conclude that reduced growth as a reaction to compensation for energy demanding regulatory mechanisms could lead to lower survival, lower reproductive potential, reduction in population size and could therefore significantly affect the ecosystem and fisheries.

4.3 Effects on otoliths

The otoliths (ear bones) of fish are made of an aragonite structure within a protein matrix and are located in the labyrinth organ of fishes. They are involved in sound detection, body orientation and acceleration based on the movement of the otoliths over sensory hairs. They are already formed during the embryonic development (Panella, 1971; Campana and Neilson, 1985; Jones, 1986). Any change in size or shape could have implications for ecological performance and individual fitness (Gagliano et al., 2008). Contrary to shells and exoskeletons of calcifying organisms, which are directly affected by chemical changes in the ambient seawater, the otoliths are protected in the inner ear of the fish. Therefore, the calcification process is dependent on the chemical

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composition of the endolymph (Borelli et al., 2003; Payan et al., 2004). In order to deposit aragonite in the protein matrix of the otoliths, the endolymph must be supersaturated with respect to aragonite (Romanek and Gauldie, 1996). Since the aragonite saturation state is correlated with the carbonate ion concentration, which is largely determined by the pH, endolymph pH regulation is needed for the aragonite crystallization of the otoliths (Takagi, 2002). Otolith growth may therefore be affected by mechanisms used to compensate extracellular pH decrease.

Checkley et al. (2009) and Munday et al. (2011a) showed that otoliths were larger in larval fish exposed to elevated pCO_2, possibly because pH regulation caused carbonate ion concentration to increase within the otolith endolymph. However, Munday et al. (2011b) found no effect on spiny damselfish (*Acanthochromis polyacanthus*) sagittal otoliths. Juvenile *Sepia officinalis* maintain calcification of the cuttlebone, a calcifying structure in the mantle of cuttlefish used for buoyancy control and functioning as an internal skeleton, under acidified conditions (up to ~6000 µatm pCO_2) (Gutowska et al., 2008) or even increase mineralization of calcium carbonate in their cuttlebones during long-term exposure to elevated pCO_2 concentrations. An increase in the size of the cuttlebones, but decreased lamellar spacing with possible negative influence on the animal's buoyancy was observed (Gutowska et al., 2010).

The reason for the different responses of the sagitta and the lapillus to an increased pCO_2 shown in our study is unknown. A likely explanation could be that the chemical composition of the endolymph is not spatially uniform. Payan et al. (1999) suggest that increasing bicarbonate and pH gradients occur from the proximal to the distal zone in the saccular endolymph of trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). Ionic gradients within the labyrinth organ might be the reason for the different responses of sagittal and lapillar otoliths.

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5 Conclusions and outlook

Even though active taxa with high metabolic rates, such as teleosts and cephalopods, have the ability to compensate acid-base disturbances actively due to their efficient ion-regulatory machinery, their embryonic stages lack specialized ion-regulatory epithelia, thus they may be the true bottleneck for ecological success (Melzner, 2009b).

The present study has shown that herring eggs can cope with increase in pCO_2, exceeding future predictions of CO_2-driven ocean acidification, but that the yolk sac larvae show a reduced protein biosynthesis capacity and therefore a potential growth reduction. Since the recruitment of fish seems to be determined during the early life stages (Koester et al., 2003; Houde et al., 2008), knowledge of the factors influencing these early developmental stages, growth and survival rates are of great importance in fisheries science. Future studies should analyse the synergistic effect of changes in temperature and CO_2 to be able to make predictions, how early life stages of fishes will react to climate induced changes.

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References

Anderson, J. T.: A review of size dependent survival during pre-recruit stages of fishes in relation to recruitment, J. Northw. Atl. Fish. Sci., 8, 55–66, 1988.

Appelhans, Y. S., Thomsen, J., Pansch, C., Melzner, F., and Martin Wahl: Sour times for benthic predators – the influence of seawater acidification on growth, feeding behaviour and acid-

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base status of *Asterias rubens* and *Carcinus maenas*, Mar. Ecol. Prog. Ser., submitted, 2011.
Belchier, M., Clemmesen, C., Cortes, D., Doan, T., Folkvord, A., Garcia, A., Geffen, A., Høie, H., Johannessen, A., Moksness, E., de Pontual, H., Ramirez, T., Schnack, D., and Sveinsbo, B.: Recruitment studies: Manual on precision and accuracy of tools, ICES Tech. Mar. Environ. Sci., 33, 35 pp., 2004.
Berge, J. A., Bjerkeng, B., Pettersen, O., Schaanning, M. T., and Oxnevad, S.: Effects of increased sea water concentrations of CO_2 on growth of the bivalve *Mytilus edulis* L., Chemosphere, 62, 681–687, 2006.
Bergeron, J. P.: Nucleic acids in ichthyoplankton ecology: a review, with emphasis of recent advances for new perspectives, J. Fish Biol., 51, 284–302, 1997.
Blaxter, J. H. S.: Herring rearing II. The effect of temperature and other factors on development, Mar. Res. Scot., 5, 19 pp., 1956.
Bodenstein, S. and Clemmesen, C.: Chloride cell distribution in early life stages in Atlantic herring (*Clupea harengus* L.), in prep., 2011.
Bonk, A. A.: Influence of the conditions of reproduction on the survival of herring embryos in the western Bering Sea, PICES 14th Annual Meeting, 181 pp., 2005.
Borelli, G., Guibbolini, M. E., Mayer-Gostan, N., Priouzeau, F., De Pontual, H., Allemand, D., Puverel, S., Tambutte, E., and Payan, P.: Daily variations of endolymph composition: relationship with the otolith calcification process in trout, J. Exp. Biol., 206, 2685–2692, 2003.
Buckley, L. J., Caldarone, E. M., and Ong, T. L.: RNA-DNA ratio and other nucleic-acid based indicators for growth and condition of marine fishes, Hydrobiol., 401, 265–277, 1999.
Buckley, L. J., Caldarone, E. M., and Clemmesen, C.: Multi-species larval fish growth model based on temperature and fluorometrically derived RNA/DNA ratios: results from a meta-analysis, Mar. Ecol. Prog. Ser., 371, 221–232, 2008.
Caldarone, E. M., Clemmesen, C., Berdalet, E., Miller, T. J., Folkvord, A., Holt, G. J., Olivar, M. P., and Suthers, I. M.: Intercalibration of four spectrofluorometric protocols for measuring RNA/DNA ratios in larval and juvenile fish, Limnol. Oceanogr.-Meth., 4, 153–163, 2006.
Caldeira, K. and Wickett, M. E.: Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean, J. Geophys. Res.-Oceans, 110, C09S04, doi:10.1029/2004jc002671, 2005.
Campana, S. E. and Neilson, J. D.: Microstructure of fish otoliths, Can. J. Fish. Aquat. Sci., 42, 1014–1032, 1985.

BGD
8, 7097–7126, 2011

Checkley, D. M., Dickson, A. G., Takahashi, M., Radich, J. A., Eisenkolb, N., and Asch, R.: Elevated CO_2 enhances otolith growth in young fish, Science, 324, 1683–1683, 2009.
Clemmesen, C.: Improvements in the fluorometric determination of the RNA and DNA content of individual marine fish larvae, Mar. Ecol. Prog. Ser., 100, 177–183, 1993.
Clemmesen, C.: The effect of food availability, age or size on the RNA/DNA ratio of individually measured herring larvae: laboratory calibration, Mar. Biol., 118, 377–382, 1994.
Clemmesen, C., Sanchez, R., and Wongtschowski, C.: A regional comparison of the nutritional condition of SW atlantic anchovy larvae, *Engraulis anchoita*, based on RNA/DNA ratios, Arch. Fish. Mar. Res., 45, 17–43, 1997.
Conway, T. and Tans, P.: NOAA/ESRL (www.esrl.noaa.gov/gmd/ccgg/trends/)
Denman, K., Christian, J. R., Steiner, N., Poertner H. O., and Nojiri, Y.: Potential impacts of future ocean acidification on marine ecosystems and fisheries: current knowledge and recommendations for future research, ICES J. Mar. Sci., 68(6), 1019–1029, 2011.
Dickson, A. G.: An exact definition of total alkalinity, and a procedure for the estimation of alkalinity and total inorganic carbon from titration data, Deep-Sea Res., 28, 609–623, 1981.
Diez, J. M. and Davenport, J.: Embryonic respiration in the spiny dogfish (*Scyliorhinus canicula* L.), J. Mar. Biol. Assoc. UK, 67, 249–261, 1987.
Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. A.: Ocean acidification: The other CO_2 problem, Annu. Rev. Mar. Sci., 1, 169–192, 2009.
Dupont, S. and Thorndyke, M. C.: Impact of CO2-driven ocean acidification on invertebrates early life-history What we know, what we need to know and what we can do, Biogeosciences Discuss., 6, 3109–3131, doi:10.5194/bgd-6-3109-2009, 2009.
Dupont, S., Lundve, B., and Thorndyke, M.: Near future ocean acidification increases growth rate of the lecithotrophic larvae and juveniles of the sea star *Crossaster papposus*, J. Exp. Zool. (Mol. Dev. Evol.), 314B, 382–389, 2010.
Ellis, R. P., Bersey, J., Rundle, S. D., Hall-Spencer, J. M., and Spicer, J. I.: Subtle but significant effects of CO_2 acidified seawater on embryos of the intertidal snail, *Littorina obtusata*, Aquat. Biol., 5, 41–48, 2009.
Fabry, V. J.: Ocean science – marine calcifiers in a high-CO_2 ocean, Science, 320, 1020–1022, 2008.
Feely, R. A., Sabine, C. L., Lee, K., Berelson, W., Kleypas, J., Fabry, V. J., and Millero, F. J.: Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans, Science, 305, 362–366, 2004.

BGD
8, 7097–7126, 2011

Feely, R. A., Sabine, C. L., Hernandez-Ayon, J. M., Ianson, D., and Hales, B.: Evidence for upwelling of corrosive "acidified" water onto the continental shelf, Science, 320, 1490–1492, 2008.
Fisher, R. A.: The design of experiments, Oliver and Boyd, Edinburgh, 1935.
Foss, A., Røsnes, B. A., and Øiestad, V.: Graded environmental hypercapnia in juvenile spotted wolffish (*Anarhichas minor* Olafsen): effects on growth, food conversion efficiency and nephrocalcinosis, Aquaculture, 220, 607–617, 2003.
Frommel, A. Y., Maneja, R., Lowe, D., Malzahn, A. M., Geffen, A. J., Folkvord, A., Piatkowski, U., and Clemmesen, C.: Ocean acidification effects on larvae of a commercially important fish species, Atlantic cod (*Gadus morhua*), Nature Climate change, submitted, 2011.
Gagliano, M., Depczynski, M., Simpson, S. D., and Moore, J. A. Y.: Dispersal without errors: Symmetrical ears tune into the right frequency for survival, Proc. R. Soc. B, 275, 527–534, 2008.
Gazeau, F., Quiblier, C., Jansen, J. M., Gattuso, J. P., Middelburg, J. J., and Heip, C. H. R.: Impact of elevated CO_2 on shellfish calcification, Geophys. Res. Lett., 34, L07603, doi:10.1029/2006gl028554, 2007.
Gooding, R. A., Harley, C. D. G., and Tang, E.: Elevated water temperature and carbon dioxide concentration increase the growth of a keystone echinoderm, P. Natl. Acad. Sci. USA, 106, 9316–9321, 2009.
Gronkjaer, P., Clemmesen, C., and St. John, M.: Nutritional condition and vertical distribution of Baltic cod larvae, J. Fish Biol., 51(A), 352–369, 1997.
Gutowska, M. A. and Melzner, F.: Abiotic conditions in cephalopod (*Sepia officinalis*) eggs: Embryonic development at low pH and high pCO_2, Mar. Biol., 156, 515–519, 2009.
Gutowska, M. A., Poertner, H. O., and Melzner, F.: Growth and calcification in the cephalopod *Sepia officinalis* under elevated seawater pCO_2, Mar. Ecol. Prog. Ser., 373, 303–309, 2008.
Gutowska, M. A., Melzner, F., Langenbuch, M., Bock, C., Claireaux, G., and Poertner, H. O.: Acid-base regulatory ability of the cephalopod (*Sepia officinalis*) in response to environmental hypercapnia, J. Comp. Physiol. B, 180, 323–335, 2010.
Havenhand, J. N., Buttler, F. R., Thorndyke, M. C., and Williamson, J. E.: Near-future levels of ocean acidification reduce fertilization success in a sea urchin, Curr. Biol., 18, R651–R652, 2008.
Hedges, L. V. and Olkin, I.: Statistical methods for meta-analysis, Academic Press, Orlando, FL, 369 pp., 1985.

BGD
8, 7097–7126, 2011

Hiroi, J., Kaneko, T., Seikai, T., and Tanaka, M.: Developmental sequence of chloride cells in the body skin and gills of Japanese flounder (*Paralichthys olivaceus*) larvae, Zool. Sci., 15, 455–460, 1998.
Houde, E. D.: Fish early life dynamics and recruitment variability, Am. Fish. Soc. Symp., 2, 17–29, 1987.
Houde, E. D.: Emerging from Hjort's Shadow, J. Northw. Atl. Fish. Sci., 41, 53–70, 2008.
Hu, M. Y., Tseng, Y. C., Stumpp, M., Gutowska, M. A., Kiko, R., Lucassen, M., and Melzner, F.: Elevated seawater pCO_2 differentially affects branchial acid-base transporters over the course of development in the cephalopod *Sepia officinalis*, Ame. J. Physiol.-Reg. I., 300, R1100–R1114, 2011.
Huwer, B., Clemmesen, C., Gronkjaer, P., and Koester, F. W.: Vertical distribution and growth performance of Baltic cod larvae – Field evidence for starvation-induced recruitment regulation during the larval stage?, Prog. Oceanogr., doi:10.1016/j.pocean.2011.04.001, 2011.
IPCC: Climate Change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change, Cambridge, United Kingdom and New York, NY, USA, 2007.
Ishimatsu, A., Kikkawa, T., Hayashi, M., Lee, K. S., and Kita, J.: Effects of CO_2 on marine fish: Larvae and adults, J. Oceanogr., 60, 731–741, 2004.
Ishimatsu, A., Hayashi, M., and Kikkawa, T.: Fishes in high-CO_2, acidified oceans, Mar. Ecol. Prog. Ser., 373, 295–302, 2008.
Jones, C.: Determining age of larval fish with the otolith increment technique, Fish. Bull. U.S., 84, 91–103, 1986.
Katoh, F., Shimizu, A., Uchida, K., and Kaneko, T.: Shift of chloride cell distribution during early life stages in seawater-adapted killifish, *Fundulus heteroclitus*, Zool. Sci., 17, 11–18, 2000.
Kikkawa, T., Ishimatsu, A., and Kita, J.: Acute CO_2 tolerance during the early developmental stages of four marine teleosts, Environ. Toxicol., 18, 375–382, 2003.
Koester, F. W., Hinrichsen, H. H., Schnack, D., St. John, M. A., Mackenzie, B. R., Tomkiewicz, J., Möllmann, C., Kraus, G., Plikshs, M., Makarchouk, A., and Aro, E.: Recruitment of Baltic cod and sprat stocks: identification of critical life stages and incorporation of environmental variability into stock-recruitment relationships, Sci. Mar., 67, 129–154, 2003.
Kroeker, K. J., Kordas, R. L., Crim, R. N., and Singh, G. G.: Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms, Ecol. Lett., 13, 1419–1434, 2010.

BGD
8, 7097–7126, 2011

Kurihara, H., Shimode, S., and Shirayama, Y.: Effects of raised CO_2 concentration on the egg production rate and early development of two marine copepods (*Acartia steueri* and *Acartia erythraea*), Mar. Poll. Bull., 49, 721–727, 2004.

Langdon, C.: Review of experimental evidence for effects of CO_2 on calcification of reef-builders, Proc. 9th Int. Coral Reef Sym., 2, 1091–1098, 2002.

Larsen, B. K., Poertner, H. O., and Jensen, F. B.: Extra- and intracellular acid-base balance and ionic regulation in cod (*Gadus morhua*) during combined and isolated exposures to hypercapnia and copper, Mar. Biol., 128, 337–346, 1997.

Leggett, W. C. and DeBlois, E.: Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stages?, Neth. J. Sea Res., 32, 119–134, 1994.

LePecq, J. B. and Paoletti, C.: A new fluorometric method for RNA and DNA determination, Anal. Biochem., 17, 100–107, 1966.

Lischka, S., Büdenbender, J., Boxhammer, T., and Riebesell, U.: Impact of ocean acidification and elevated temperatures on early juveniles of the polar shelled pteropod *Limacina helicina*: mortality, shell degradation, and shell growth, Biogeosciences, 8, 919–932, doi:10.5194/bg-8-919-2011, 2011.

Malzahn, A. M., Clemmesen, C., Wiltshire, K. H., Laakmann, S., and Boersma, M.: Comparative nutritional condition of larval dab *Limanda limanda* and lesser sandeel *Ammodytes marinus* in a highly variable environment, Mar. Ecol. Prog. Ser., 334, 205–212, 2007.

McGurk, M. D.: Allometry of herring mortality, Trans. Am. Fish. Soc., 122, 1035–1042, 1993.

Melzner, F., Gobel, S., Langenbuch, M., Gutowska, M. A., Poertner, H. O., and Lucassen, M.: Swimming performance in atlantic cod (*Gadus morhua*) following long-term (4–12 months) acclimation to elevated seawater pCO_2, Aquat. Toxicol., 92, 30–37, 2009a.

Melzner, F., Gutowska, M. A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M. C., Bleich, M., and Pörtner, H.-O.: Physiological basis for high CO2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny?, Biogeosciences, 6, 2313–2331, doi:10.5194/bg-6-2313-2009, 2009.

Metzger, R., Sartoris, F. J., Langenbuch, M., and Poertner, H. O.: Influence of elevated CO_2 concentrations on thermal tolerance of the edible crab *Cancer pagurus*, J. Therm. Biol., 32, 144–151, 2007.

Michaelidis, B., Spring, A., and Poertner, H. O.: Effects of long-term acclimation to environmental hypercapnia on extracellular acid-base status and metabolic capacity in mediterranean fish *Sparus aurata*, Mar. Biol., 150, 1417–1429, 2007.

BGD
8, 7097–7126, 2011

Munday, P. L., Donelson, J. M., Dixson, D. L., and Endo, G. G. K.: Effects of ocean acidification on the early life history of a tropical marine fish, P. Roy. Soc. B-Biol. Sci., 276, 3275–3283, 2009.
Munday, P. L., Hernaman, V., Dixson, D. L., and Thorrold, S. R.: Effect of ocean acidification on otolith development in larvae of a tropical marine fish, Biogeosciences, 8, 1631–1641, doi:10.5194/bg-8-1631-2011, 2011a.
Munday, P. L., Gagliano, M., Donelson, J. M., Dixson, D. L., and Thorrold, S. R.: Ocean acidification does not affect the early life history development of a tropical marine fish, Mar. Ecol. Prog. Ser., 423, 211–221, 2011b.
Nakagawa, S. and Foster, T. M.: The case against retrospective statistical power analyses with an introduction to power analysis, Acta. Ethol., 7, 103–108, 2004.
Panella, G.: Fish otolith: Daily layers and periodical patterns, Science, 173, 1124–1127, 1971.
Payan, P., Edeyer, A., De Pontual, H., Borelli, G., Boeuf, G., and Mayer-Gostan, N.: Chemical composition of saccular endolymph and otolith in fish inner ear: Lack of spatial uniformity, Am. J. Physiol.-Reg. I., 277, R123–R131, 1999.
Payan, P., De Pontual, H., Boeuf, G., and Mayer-Gostan, N.: Endolymph chemistry and otolith growth in fish, C. R. Palevol, 3, 535–547, 2004.
Pepin, P., Evans, G. T., and Shears, T. H.: Patterns of RNA/DNA ratios in larval fish and their relationship to survival in the field, ICES J. Mar. Sci., 56, 697–706, 1999.
Pierrot, D., Lewis, E., and Wallace, D. W. R.: MS Excel program developed for CO_2 system calculations. Macro for low salinities, Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee, 2006.
Poertner, H. O. and Farrell, A. P.: Physiology and climate change, Science, 322, 690–692, 2008.
Poertner, H. O. and Peck, M. A.: Climate change effects on fishes and fisheries: Towards a cause-and-effect understanding, J. Fish Biol., 77, 1745–1779, 2010.
Poertner, H. O., Langenbuch, M., and Reipschlaeger, A.: Biological impact of elevated ocean CO_2 concentrations: Lessons from animal physiology and earth history, J. Oceanogr., 60, 705–718, 2004.
Poertner, H. O., Langenbuch, M., and Michaelidis, B.: Synergistic effects of temperature extremes, hypoxia, and increases in CO_2 on marine animals: From earth history to global change, J. Geophys. Res.-Oceans, 110, C09S10, doi:10.1029/2004jc002561, 2005.
Raven, J., Caldeira, K., Elderfield, H., Hoegh-Guldberg, O., Liss, P. S., Riebesell, U., Shepherd,

BGD
8, 7097–7126, 2011

J., Turley, C., and Watson, A. J.: Ocean acidification due to increasing atmospheric carbon dioxide, Policy Document 12/05, London: The Royal Society, 60 pp., 2005.
Riebesell, U.: Effects of CO_2 enrichment on marine phytoplankton, J. Oceanogr., 60, 719–729, 2004.
Riebesell, U., Fabry, V. J., Hansson, L., and Gattuso, J.-P.: Guide to best practices for ocean acidification research and data reporting, Luxembourg: Publications Office of the European Union., 260 pp., 2010.
Ries, J. B., Cohen, A. L., and McCorkle, D. C.: Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification, Geology, 37, 1131–1134, 2009.
Romanek, C. S. and Gauldie, R. W.: A predictive model of otolith growth in fish based on the chemistry of the endolymph, Comp. Biochem. Physiol. A, 114, 71–79, 1996.
Rosenthal, H. and Alderdice, D. F.: Sublethal effects of environmental stressors, natural and pollutional, on marine fish eggs and larvae, J. Fish. Res. Board Can., 33, 2047–2065, 1976.
Roy, R. N., Roy, L. N., Vogel, K. M., Portermoore, C., Pearson, T., Good, C. E., Millero, F. J., and Campbell, D. M.: The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45°C, Mar. Chem., 44, 249–267, 1993.
Sasai, S., Kaneko, T., and Tsukamoto, K.: Extrabranchial chloride cells in early life stages of the Japanese eel, *Anguilla japonica*, Ichthyol. Res., 45, 95–98, 1998.
Shiraishi, K., Kaneko, T., Hasegawa, S., and Hirano, T.: Development of multicellular complexes of chloride cells in the yolk-sac membrane of tilapia (*Oreochromis mossambicus*) embryos and larvae in seawater, Cell Tissue Res., 288, 583–590, 1997.
Shirayama, Y. and Thornton, H.: Effect of increased atmospheric CO_2 on shallow water marine benthos, J. Geophys. Res.-Oceans, 110, C09S08, doi:10.1029/2004jc002618, 2005.
Sprague, J. B.: Measurement of pollutant toxicity to fish III. Sublethal effects and "safe" concentrations, Water Res., 5, 245–266, 1971.
Stoll, M. H. C., Bakker, K., Nobbe, G. H., and Haese, R. R.: Continuous-flow analysis of dissolved inorganic carbon content in seawater, Anal. Chem., 73, 4111–4116, 2001.
Takagi, Y.: Otolith formation and endolymph chemistry: a strong correlation between the aragonite saturation state and pH in the endolymph of the trout otolith organ, Mar. Ecol. Prog. Ser., 231, 237–245, 2002.
Thomsen, J., Gutowska, M. A., Saphörster, J., Heinemann, A., Trübenbach, K., Fietzke, J., Hiebenthal, C., Eisenhauer, A., Körtzinger, A., Wahl, M., and Melzner, F.: Calcifying invertebrates succeed in a naturally CO2-rich coastal habitat but are threatened by high levels of

BGD
8, 7097–7126, 2011

future acidification, Biogeosciences, 7, 3879–3891, doi:10.5194/bg-7-3879-2010, 2010.
Voss, R., Clemmesen, C., Baumann, H., and Hinrichsen, H. -H.: Baltic sprat larvae: Coupling food availability, larval condition and survival, Mar. Ecol. Prog. Ser., 308, 243–254, 2006.
Wood, H. L., Spicer, J. I., and Widdicombe, S.: Ocean acidification may increase calcification rates, but at a cost, P. Roy. Soc. B-Biol. Sci, 275, 1767–1773, 2008.

BGD
8, 7097–7126, 2011

Table 1. Seawater carbonate system speciation for the different treatment levels during the course of the experiment. Variables were calculated using measured pH_F, A_T, salinity (14.0) and temperature (13.6 ± 0.4 °C) of the respective replicates at the beginning, in the middle and at the end of the experiment. Values are means ±SD.

Treatment	pH_F (free scale)	A_T [µmol kg^{-1}]	C_T [µmol kg^{-1}]	pCO_2 [µatm]	CO_2 [µmol kg^{-1}]	HCO_3^- [µmol kg^{-1}]	CO_3^{2-} [µmol kg^{-1}]	Ω_{arag}
1 (control)	8.08 ± 0.07	2070.2 ± 4.1	1989.9 ± 20.5	480 ± 81	21.5 ± 3.4	1887.8 ± 28.2	80.6 ± 11.2	1.27 ± 0.17
2	7.67 ± 0.07	1965.8 ± 4.7	1981.2 ± 15.4	1260 ± 218	55.7 ± 8.8	1894.1 ± 11.2	31.5 ± 4.3	0.49 ± 0.07
3	7.49 ± 0.05	1922.6 ± 5.1	1977.4 ± 14.9	1859 ± 240	81.6 ± 9.0	1874.9 ± 8.2	20.9 ± 1.9	0.33 ± 0.03
4	7.33 ± 0.03	1870.2 ± 4.1	1967.4 ± 7.6	2626 ± 197	115.4 ± 6.6	1837.9 ± 3.8	14.1 ± 0.7	0.22 ± 0.01
5	7.28 ± 0.03	1854.8 ± 3.1	1967.1 ± 8.0	2903 ± 204	128.5 ± 7.3	1826.2 ± 3.0	12.5 ± 0.6	0.20 ± 0.01
6	7.05 ± 0.03	1737.5 ± 4.9	1934.4 ± 16.6	4635 ± 340	206.0 ± 18.5	1721.5 ± 4.0	6.9 ± 0.7	0.11 ± 0.01

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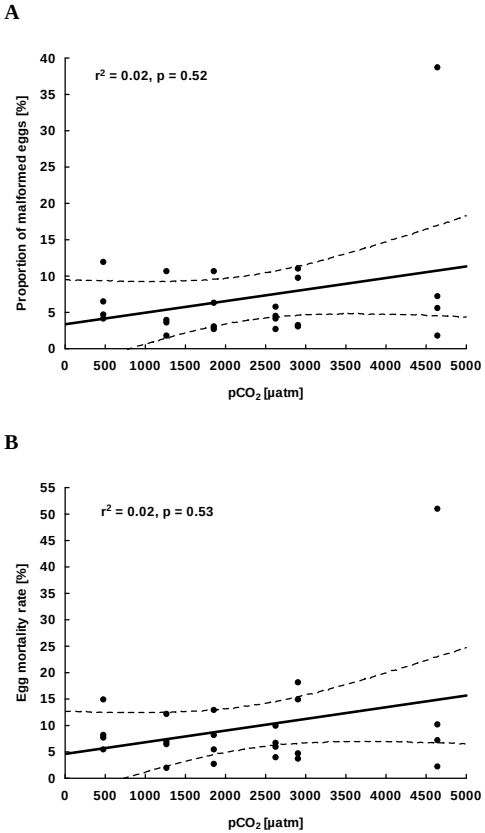

Fig. 1. (A) Proportion of malformed eggs and **(B)** mortality rate during the embryonic development depending on the pCO_2 treatment level (4 replicates each). Data points are percentages of malformed eggs and mortality rates, respectively, of incubation plate 1 of the respective replicate. The solid line shows the regression line, whereas the dashed lines represent the 95 % confidence intervals. The r^2 and P-value were derived from log transformed data.

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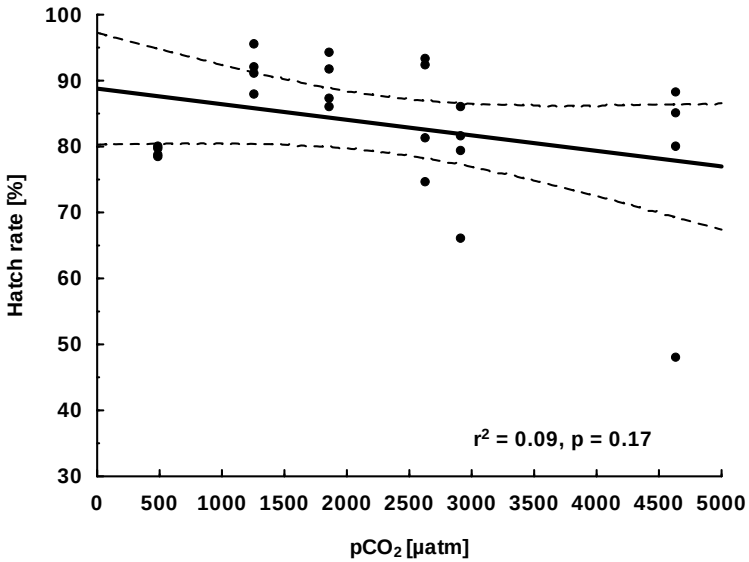

Fig. 2. Hatch rate (%) of Atlantic herring eggs depending on the pCO_2 condition (4 replicates each). Data points are percentages of hatched larvae of incubation plate 1 of the respective replicate. The solid line shows the regression line, whereas the dashed lines represent the 95 % confidence intervals. The r^2 and P-value were derived from arcsine transformed data.

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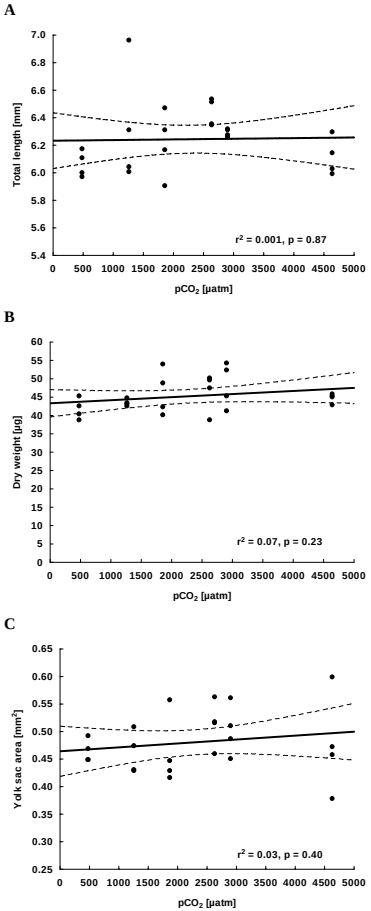

Fig. 3. **(A)** Total length, **(B)** dry weight and **(C)** yolk sac area of newly hatched Atlantic herring larvae displayed against the pCO_2 treatment levels (4 replicates each). Data points are mean values of 6 individual larvae. The solid line shows the regression line, whereas the dashed lines represent the 95 % confidence intervals. **(A)** The r^2 and P-value were derived from log transformed data.

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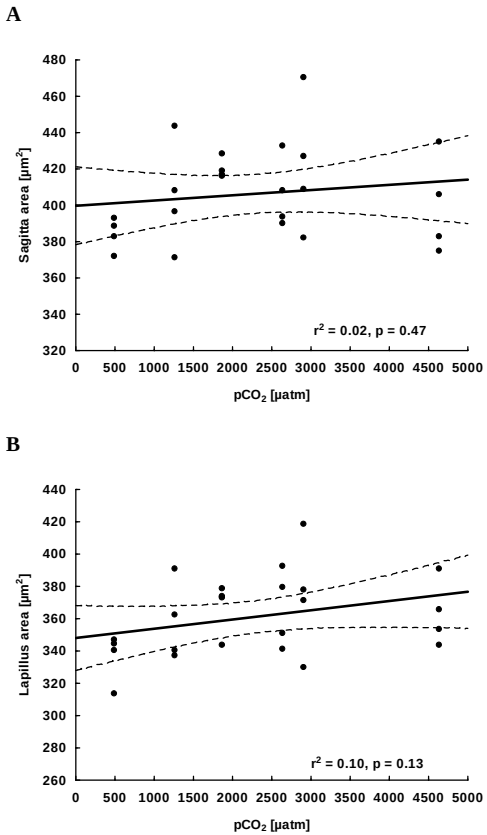

Fig. 4. (A) Sagitta area and **(B)** lapillus area of Atlantic herring larvae at hatch depending on the pCO_2 treatment level (4 replicates each). Data points are mean values of 3 individual larvae (6 otoliths). The solid line shows the regression line, whereas the dashed lines represent the 95 % confidence intervals.

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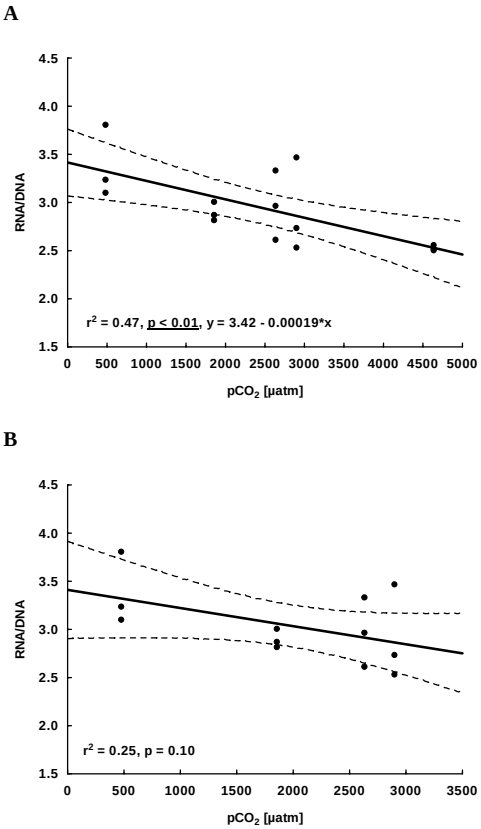

Fig. 5. **(A)** RNA/DNA ratio of newly hatched Atlantic herring larvae across the entire pCO_2 gradient and **(B)** without the highest treatment level. Due to accidental loss of samples larvae of only 3 replicates per treatment level could be used for nucleic acid determination. Furthermore, nucleic acids could not be examined for treatment level 2. Data points are mean values of 6 individual larvae. The solid line shows the regression line, whereas the dashed lines represent the 95 % confidence intervals.

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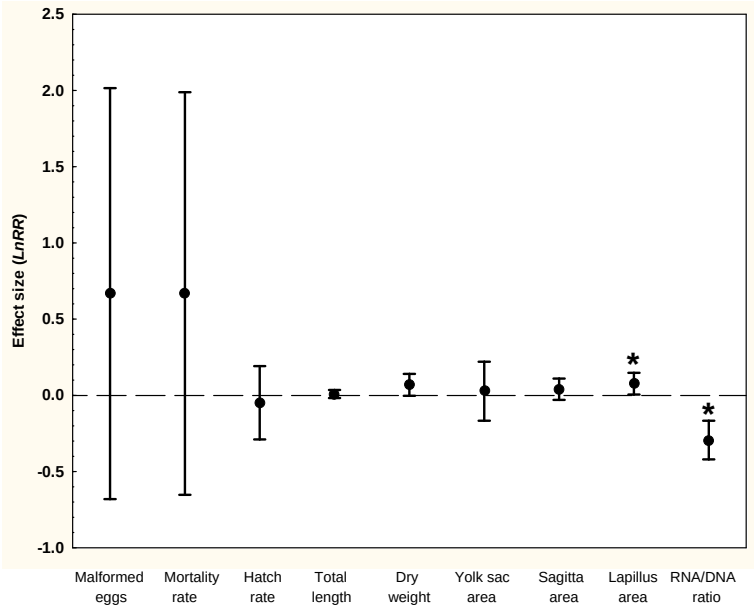

Fig. 6. Effect sizes and 95 % confidence intervals for all examined variables (embryonic malformations, mortality rate of eggs, hatch rate and total length, dry weight, yolk sac area, sagitta area, lapillus area and RNA/DNA ratio of newly hatched Atlantic herring larvae) based on the differences between control (pCO_2 = 480 µatm) and highest treatment (pCO_2 = 4635 µatm). The effect size is significant when the 95 % confidence interval does not overlap with zero (*).

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