Journal of Experimental Marine Biology and Ecology 466 (2015) 42–48

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

The swimming kinematics and foraging behavior of larval Atlantic herring (*Clupea harengus* L.) are unaffected by elevated pCO_2

Rommel H. Maneja [a,*], Andrea Y. Frommel [a], Howard I. Browman [b], Audrey J. Geffen [c], Arild Folkvord [c], Uwe Piatkowski [a], Caroline M.F. Durif [b], Reidun Bjelland [b], Anne Berit Skiftesvik [b], Catriona Clemmesen [a]

[a] Helmholtz-Zentrum für Ozeanforschung Kiel-GEOMAR, Düsternbrooker Weg 20, 24105 Kiel, Germany
[b] Institute of Marine Research, Austevoll Research Station, 5392 Storebø, Norway
[c] Department of Biology, University of Bergen, PO Box 7803, N-5020 Bergen, Norway

ARTICLE INFO

Article history:
Received 17 June 2014
Received in revised form 10 February 2015
Accepted 11 February 2015
Available online xxxx

Keywords:
Atlantic herring larva
Elevated seawater pCO_2
Foraging behavior
Swimming behavior

ABSTRACT

The kinematics of swimming behavior of Atlantic herring larvae cultured under three pCO_2 conditions (control — 370, medium — 1800, and high — 4200 μatm) were extracted at 34 days post-hatch (dph) from swim path recordings obtained using silhouette video photography. The swim paths were analyzed for move duration, speed and length, stop duration, and horizontal and vertical turn angles to determine the effects of elevated pCO_2 on fish larval behavior. The swimming kinematics and occurrence of S-postures in Atlantic herring larvae that had survived to 34-dph were unaffected by extremely elevated levels of seawater pCO_2, indicating that at least some larvae in the population are resilient to ocean acidification.

1. Introduction

Mortality in broadcast-spawning marine fishes is high, with a severe decrease in abundance from the embryonic through to the juvenile stage (Houde, 2002). Major processes affecting mortality during the early life stages of fish include predation, starvation, disease, and physical environmental processes (Folkvord et al., 2009; Fuiman, 1989; Haslob et al., 2009; Peck et al., 2012b). As fish larvae grow, sensory and swimming abilities develop and are associated with improved prey location and capture success (Hauss and Peck, 2009) and predator avoidance and evasion (Fuiman, 1989). Fish larvae also modulate their swimming behavior in response to variation in prey availability, light and turbulence (Batty, 1987; MacKenzie and Kiørboe, 1995; Munk and Kiørboe, 1985; Peck et al., 2012a).

Ocean acidification might increase mortality in marine fish larvae through, for example, effects on their behavior that make them more susceptible to predation, reduce their food intake, or alter their orientation towards nursery grounds. Disruptions in the behavior of the larvae and juveniles of tropical marine fish species have been reported, including impaired olfaction resulting in changes in predator/prey detection and avoidance (Cripps et al., 2011; Dixson et al., 2010; Munday et al., 2010) and loss of homing ability (Munday et al., 2009). These behavioral changes have been attributed to a pCO_2-related disruption of neurotransmitter function (Nilsson et al., 2012). Additional behavioral changes include loss of lateralization (Domenici et al., 2012; Nilsson et al., 2012) and disruptions in learning anti-predator responses, among other behavioral problems (Ferrari et al., 2012; Hamilton et al., 2014; Pimentel et al., 2014).

Atlantic herring is an important commercial fish species in the North Atlantic and is also a major food source for other species (Nash et al., 2009; Payne et al., 2009). It is widely distributed, spawns over extended periods, and produces eggs and larvae that are adapted to a broad range of temperature, light, hydrographic conditions, predator fields, and food availability (e.g. Geffen, 2009). Some herring populations may be more vulnerable to ocean acidification, especially those spawned in well-ventilated and highly-flushed benthic environments that are more likely to be affected by anthropogenic pCO_2 in the near future. Shallowing of the aragonite saturation horizon and decrease in surface pH (Gangstø et al., 2008; Olafsson et al., 2009; Steinacher et al., 2009) could expose the eggs at some benthic spawning grounds and pelagic larvae to high pCO_2 conditions.

In this study, we investigated the possible effects of elevated pCO_2 on the frequency of S-postures and the swimming kinematics (move duration, speed and length, stop duration, and turn angles) of Atlantic herring larvae. The swimming kinematics of fish larvae, which describe the changes in position of the larvae without reference to hydrodynamic forces (Videler, 1993), can be viewed as proxies for changes in behavior that occur in response to elevated pCO_2. The aim of the study was to analyze the behavioral responses resulting from an increase in CO_2

* Corresponding author at: Center for Environment and Water, Research Institute, King Fahd University of Petroleum and Minerals, 31261 Dhahran, Saudi Arabia. Tel.: +966 3 860 7794.
E-mail address: rmaneja@kfupm.edu.sa (R.H. Maneja).

http://dx.doi.org/10.1016/j.jembe.2015.02.008
0022-0981/

levels at a stage where sufficient swimming performance is already achieved (flexion of the notochord) and at a developmental time when the gill lamellae, the sites where acid base regulation mostly takes place, is not fully developed yet (see Frommel et al., 2014).

2. Materials and methods

2.1. Seawater pCO_2 manipulation

An experiment with three pCO_2 levels (control: 370 μatm, medium: 1800 μatm, high: 4200 μatm) and three replicates was conducted in the land-based mesocosms at the University of Bergen's Espegrend Marine Station from March to May 2010. A low pCO_2 (700 μatm) treatment was initially planned but mechanical error in the setup at the start of the experiment resulted in the loss of this treatment (Frommel et al., 2014). A flow-through system supplied seawater pumped from a 40-meter deep water intake into 2300-L, 1.5-m deep tanks. CO_2 was introduced into the bottom of the tanks by bubbling through diffusers. The diffusers were placed close to the water inflow in order to facilitate efficient CO_2 diffusion and water circulation. Seawater pH values (average ± standard deviation) measured during the experiment were: control, 8.08 ± 0.01; medium, 7.44 ± 0.02; and high, 7.08 ± 0.02. pCO_2 values (average ± standard deviation) were: control, 366.7 ± 7.1 μatm; medium, 1877.7 ± 74.2 μatm; and high, 4247.7 ± 269.0 μatm. Short term variation in water temperature was minimized by placing the replicate tanks inside water baths. Water temperature was about 5 °C in March and increased to 10 °C by the end of May. Dissolved oxygen concentration was above 90% saturation and mean salinity was at 33.3 psu. For further details, see (Frommel et al. (2012) and Maneja et al. (2013)).

2.2. Larval rearing

Ripe adult herring from Lindåspollene in western Norway was strip-spawned onto glass plates. The plates were then placed into the middle of each tank on March 24, 2010. To avoid possible parental effects, fertilized eggs from 10 pairs of mature male and female herring were distributed in all the tanks. The pH of the tanks was adjusted to the target treatment pH levels at four days post-fertilization. Prior to the onset of hatching, the glass plates were suspended inside floating buckets in order to collect the newly-hatched larvae. Day 0 post-hatching date (dph) was set when 50% of the eggs had hatched (April 16, 2010 = 0-dph). The newly-hatched larvae from the same treatment were collected and redistributed to the replicate tanks at a stocking density of 4 larvae L^{-1}. Atlantic cod (*Gadus morhua* L.) larvae were stocked at a similar initial density and co-reared in each tank with the herring larvae throughout the experiment (Maneja et al., 2013). The newly hatched herring larvae were distributed into the tanks seven days after the newly hatched cod larvae had been introduced. At this time, a vertical separation between the cod and herring larvae in the 2300-L tanks was already observed reducing the competition for food due to size and spatial separation.

Live natural zooplankton composed mostly of copepod nauplii and adults was filtered daily from the adjacent seawater using a Hydrotech size-selective filter system to provide a feeding density of 2000 prey L^{-1} (Seljeset et al., 2010). Larvae were fed initially with 80–250 μm-sized zooplankton, which was gradually increased to 350–500 μm by 27-dph. Field surveys demonstrated that Atlantic herring larvae 5–45 mm in standard length prey on various developmental stages and adults of several copepod species with mean carapace widths of ca. 80–500 μm (Cohen and Lough, 1983).

The larvae were exposed to a natural light cycle and intensity. During sunny days with no cloud cover, average light intensities, measured using a LI-COR Underwater Quantum Sensor, were 330 and 93.6 μmol s^{-1} m^{-2} at 5-cm below the water surface and at the bottom of the tanks, respectively.

2.3. Ethics statement

This study was carried out in strict accordance with the laboratory regulations applicable in 2010, which are laid down in the Animal Welfare Act (LOV 2009-06-19 nr 97: Lov om dyrevelferd). Norway has entered into international agreements undertaken to follow EU Directive on laboratory animals (86/609/EEC) and the Council of Europe Convention on laboratory animals (ETS 123). The protocol was approved by the national regulatory Committee on the Ethics of Animal Experiments (Forsøksdyrutvalget) with Permit Number: ID2346. All conditions and sampling were conducted to minimize suffering.

2.4. Larval swimming behavior

A 3-dimensional silhouette video photography system (SVP) was used to observe the swimming behavior of 34-dph Atlantic herring larvae (see Browman et al., 2003 for a complete description of the equipment). The sizes (i.e. standard length, defined as the distance from the snout to the base of the caudal fin) of fish observed for swimming and foraging behavior at 34-dph were not measured. Instead, mean fish sizes per treatment were interpolated from the daily growth rates between 32 and 39-dph (Frommel et al., 2014). Approximately 60 larvae per tank were collected and placed in floating buckets inside each tank on the night prior to video recording. The floating buckets had a mesh bottom to keep live zooplankton out and provide adequate water exchange. At around 0400 h the following day, cylindrical transparent plastic bags containing 7 L of seawater from each tank were used to transport the larvae to the Institute of Marine Research's Austevoll Research Station. To keep the water temperature stable, the plastic bags were placed inside coolers. The larvae were not fed and were kept in the dark until the video recording in order to increase the probability that they would forage for food during the observation period. Transport of fish larvae from Espegrend to Austevoll Research Station was previously reported to have no detrimental effects on the larvae (Vollset et al., 2011).

The seawater containing the herring larvae was carefully transferred into separate 20 × 20 × 20-cm observation tanks inside the dark, temperature-controlled SVP room. Surface or edge effect on the swimming behavior of recorded larvae was avoided by screening the outer 5 cm of the observation tanks ensuring that no observations were made near a barrier. Air temperature inside the room was adjusted to match the water temperature in the mesocosm tanks. The observation tanks were sealed with plastic covers, which were tightly secured with elastic bands, to reduce efflux of CO_2. Diffusion of CO_2 from the seawater was previously estimated from the highest pCO_2 treatment to be around 360 μatm, and did not result in the convergence of pH and pCO_2 levels after 8 h without CO_2-bubbling (Maneja et al., 2013).

The recording sequence of replicate tanks was High-R1, Control-R3, Medium-R3, Control-R2, High-R2, Medium-R2, Medium-R1, Control-R1 and High-R3. Fresh zooplankton at a density of 3000 prey L^{-1}, and composed mainly of copepod nauplii and some adult copepods were introduced into each observation tank 10 min prior to recording. The recording duration for each replicate was 30 min and the temporal resolution of the video was 25 frames per second.

2.5. Analysis of swim paths

Larval swim paths were reconstructed using the TrakFish software (Racca Scientific Consulting and JASCO Research Ltd., Victoria, British Columbia, Canada) (Browman et al., 2003). In TrakFish, the two orthogonally-oriented views of the experimental tank were calibrated by creating a reference volume from four marks with known coordinates recorded against each front of the observation tank facing the camera. The reference volume established a scaled coordinate system from which the three-dimensional spatial coordinates of the fish's location in every frame were determined. To ensure representative

sampling, swim paths were reconstructed frame-by-frame from the initial, middle and last 5 min of the observation period.

Using the Anapaths software (Racca Scientific Consulting and JASCO Research Ltd., Victoria, British Columbia, Canada) (Browman et al., 2003), paths that were closely adjacent to each other and which were consistent with a single swim path were joined together. Paths that were too short (below 2 body lengths) and/or had extremely jagged (unrealistic) swim trajectories were not included in the analysis. The kinematic variables of the swim paths – move duration, speed and length, stop duration, and horizontal and vertical turn angles (i.e. change in direction after a stop in the horizontal and vertical planes) – were extracted using the Anapaths software. The output files for each replicate tank consisted of a list of recorded variables – for example – turn angles, which did not differentiate individual fish in the tank. For statistical analysis, all data points from all reconstructed fish swim path were used as observation values (Maneja et al., 2013).

2.6. Analysis of foraging behavior

Upon locating possible prey items, herring larvae coil themselves into a sinusoidal posture (termed an S-posture) and move towards the particle by sculling the pectoral fins (described in, for example, Munk and Kiørboe, 1985; Rosenthal and Hempel, 1970; Beyer, 1980). S-postures are either released with a lunge towards the prey particle (here termed a completed S-posture) or the S-posture is relaxed without a lunge towards the putative prey particle (here termed an aborted S-posture — sensu Checkley, 1982; Browman and O'Brien, 1992a,b) and the larva continues swimming. Whether a completed S-posture resulted in a successful capture of prey could not be determined because the magnification used to record the swimming behavior did not provide high enough resolution to identify prey items. Nonetheless, since all earlier reports of S-postures in herring larvae are consistent with them being associated with active prey search and feeding, we interpreted the occurrence of S-postures as being indicative of foraging activity, with those ending in a lunge towards the particle assumed to be feeding attempts and those that were released assumed to be aborted feeding attempts.

The foraging behavior of herring larvae was evaluated by observing twelve equally-spaced one-minute video segments (from the 30 min recorded) from each replicate tank viewed from one angle. Five larvae were randomly selected from each one minute video segment and were followed until they disappeared from the video screen. The number of S-postures that they assumed, and whether these were completed or aborted, was recorded. Since larvae moved in and out of the observation area, it was not possible to determine whether the same larva was being observed more than once during the 12 one minute observation periods. Therefore, each one minute observation was treated as an independent observation, even though an individual fish may have been observed more than once. Following from this, the frequency of S-postures (number of S-postures per minute), and the ratio between those that were completed vs. aborted, were calculated.

2.7. Data analysis

The horizontal and vertical turn angle components of the swim paths were analyzed using circular statistics (Batschelet, 1981). A non-parametric Mardia–Watson Wheeler test (MWW test) was used to compare distributions of the turn angles between treatments. The stop duration, move duration, move speed and move length data did not meet the assumptions of homogeneity of variance, showed highly skewed distributions and an unbalanced number of values per treatment. Therefore, a non-parametric two-sample Kolmogorov–Smirnov (KS) test was applied to compare distributions between treatments. Values were pooled between replicates and binned so that the number of intervals was less than 50. A 5% significance level was used in all tests. The frequency of S-postures in the different treatments was compared using two-way ANOVA with pCO_2 treatment and the sequence in which the observations were made as factors. A chi-square analysis of contingency was used to compare the proportions of completed and aborted S-postures in the different pCO_2 treatments.

3. Results

3.1. Larval standard length

Elevated pCO_2 resulted in smaller larvae throughout the duration of the experiment (Frommel et al., 2014). The estimated mean larval standard lengths at the time of the swimming behavior observations (34-dph) –interpolated from the daily growth rate between 32 and 39-dph –were: 17.7 mm in the control, 16.7 mm in the medium, and 15.7 mm in the high treatment.

3.2. Swimming behavior

There were no significant differences in the distribution of move duration, move length, move speed, or stop duration between pCO_2 treatments (Table 1). There were no patterns in the distributions as a function of time of recording (Fig. 1).

Turn angles were not significantly affected by elevated pCO_2 (Fig. 2). The median turn angles ranged from 17° to 20° and 68° to 71° for vertical and horizontal turns, respectively (Table 2). The horizontal turn angles were distributed almost uniformly from 0 to 180° while most of the vertical turn angles were less than 45°.

3.3. Observations of foraging behavior

The frequency with which herring larvae took up S-postures was unaffected by pCO_2 treatment and this was not related to the timing of the observations — Nested ANOVA, $F_{treatment}$ (2, 72) = 1.0674, p = 0.4349, $F_{time(treatment)}$ (33, 72) = 1.03, p = 0.0321, N = 108 (Fig. 3A). The proportion of completed S-postures relative to the total number of S-postures observed (control: 0.50; medium: 0.44; high: 0.40) did not differ significantly between pCO_2 treatments (chi-square = 1.570, df = 2, p = 0.456) (Fig. 3B).

4. Discussion

Results indicate that the swimming performance and feeding ability of late-stage herring larvae will be unaffected by near-future levels of ocean acidification, which is projected to reach 850 μatm by the end of the century (IPCC, 2007). The pCO_2 concentrations used in the study were chosen to challenge the physiological limits of the fish larvae across a wide range of pCO_2 perturbation. Exposure of the early life stages of fish to extreme pCO_2 concentration is possible particularly in areas where upwelling events occur and in high latitude seas, which are highly vulnerable to ocean acidification and projected to be the first areas to experience its effects (Bates et al., 2009; Fabry et al., 2009; Steinacher et al., 2009). For example, Atlantic herring has a natural spawning ground in the Kiel Fjord (Franke and Clemmesen, 2011), where upwelling events can bring seawater of >2300 μatm pCO_2 up to the surface (Thomsen et al., 2010).

The swimming kinematics of Atlantic herring larvae tested at 34-dph were not significantly affected by extremely elevated levels of pCO_2. The median swimming speed of the herring larvae (15.7–17.7 mm mean length) that we observed in this experiment ranged from 14.7 to 16.1 mm s^{-1}. This is consistent with the swimming speeds reported in earlier studies for herring larvae of various sizes: 10–11 mm s^{-1} in 11–15 mm larvae and 21–25 mm s^{-1} in 19–24 mm larvae (Rosenthal, 1968). The swimming kinematics of Atlantic cod larvae (*G. morhua*) observed from the same rearing experiment were also unaffected by elevated pCO_2 (Maneja et al., 2013), and similar results have been reported for walleye pollock, another cold-water species with pelagic larvae (Hurst et al., 2012, 2013).

Table 1
Effect of different pCO_2 treatments on the swimming kinematics of 34-day post-hatch Atlantic herring (*Clupea harengus* L.) larvae. Differences in the distributions of the variables between treatments were tested using Kolmogorov–Smirnov two-sample tests. pCO_2 treatments: C = control, M = medium, H = high, NS = non-significant difference.

Linear variable	Treatment	No. of bins	Median	Min–max	Kolmogorov–Smirnov test: Max D	Conclusion
Move duration	C	19	0.24	0.12–2.16	C vs. M: 0.11	NS
	M	19	0.24	0.12–1.92	M vs. H: 0.16	NS
	H	19	0.24	0.12–1.68	H vs. C: 0.16	NS
Move speed	C	43	16.1	7.5–107	C vs. M: 0.02	NS
	M	43	14.7	7.5–86.3	M vs. H: 0.12	NS
	H	43	15.9	7.5–89.9	H vs. C: 0.23	NS
Move length	C	20	4.71	0.9–59.2	C vs. M: 0.25	NS
	M	20	3.91	0.9–52.3	M vs. H: 0.15	NS
	H	20	4.55	0.9–55.4	H vs. C: 0.25	NS
Stop duration	C	19	0.12	0.12–3.84	C vs. M: 0.56	NS
	M	19	0.12	0.12–2.28	M vs. H: 0.16	NS
	H	19	0.12	0.12–1.56	H vs. C: 0.11	NS

Neither the frequency of S-postures taken up by herring larvae nor the proportions of completed and aborted S-postures were affected by elevated pCO_2. This suggests that herring larvae in the elevated pCO_2 treatments were foraging normally relative to the larvae from the control group. In anchovy larvae, failure to complete a feeding sequence was caused by the larva's inability to closely approach the prey while forming the S-posture, which resulted in only 40% of S-postures being completed (Hunter, 1972). The proportion of S-postures that were completed in herring larvae varied with food density, ranging from 40% at less than 15 prey L^{-1} to 70% at 360 prey L^{-1} (Munk and Kiørboe, 1985). Aborted S-postures in white crappie (*Pomoxis annularis*) larvae ranged from about 15 to 70% relative to total S-postures, with the percentage of aborted S-postures decreasing with fish size (Browman and O'Brien, 1992a). In our study, prey density was similar in all treatments and completed S-postures ranged from 18 to 65% of all S-postures observed — this proportion of completed S-postures is consistent with earlier studies on herring larvae.

Our experiment was not designed to observe larval "choices", as was the case in several other studies on the effect of elevated pCO_2 on fishes (e.g. Cripps et al., 2011; Dixson et al., 2010; Domenici et al., 2012; Ferrari et al., 2012; Munday et al., 2009, 2010; Nilsson et al., 2012), but rather to measure alternate behavioral endpoints such as the (clearly ecologically relevant) kinematics of swimming and foraging behavior. Thus, we cannot say whether or not there would be pCO_2-related differences in the behavior of herring larvae subjected to "choice experiments". A recent report (Jutfelt et al., 2013) showed behavioral modifications in three-spined stickleback, however, they did not observe concomitant abnormalities in swimming behavior. It is, therefore, possible that

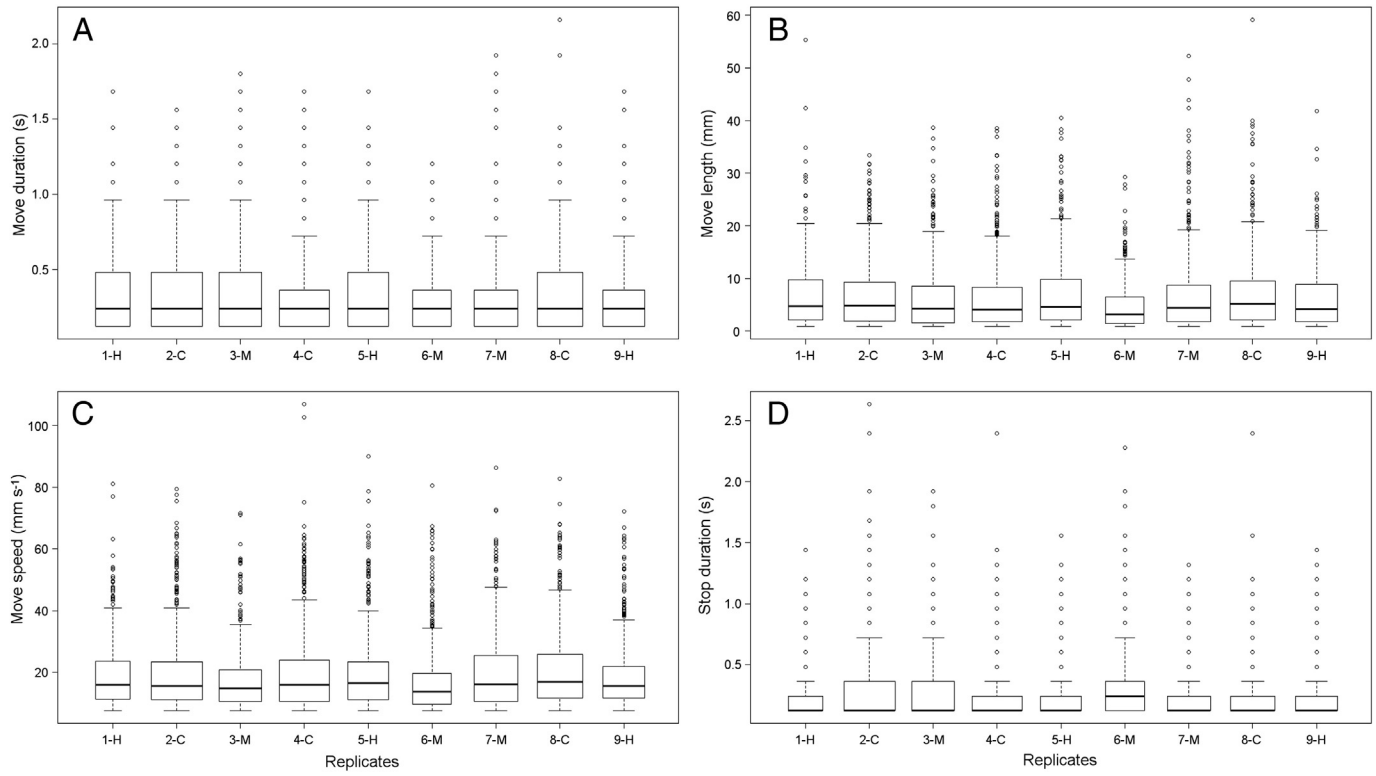

Fig. 1. Effect of pCO_2 treatment on the move duration (A), move length (B), move speed (C), and stop duration (D) of 34-day post-hatch Atlantic herring (*Clupea harengus* L.) larvae. The widths of the boxes are proportional to the number of observations (n). The replicates are presented along the x-axis in the sequence in which the observations were made (1–9). C = control, M = medium, H = high.

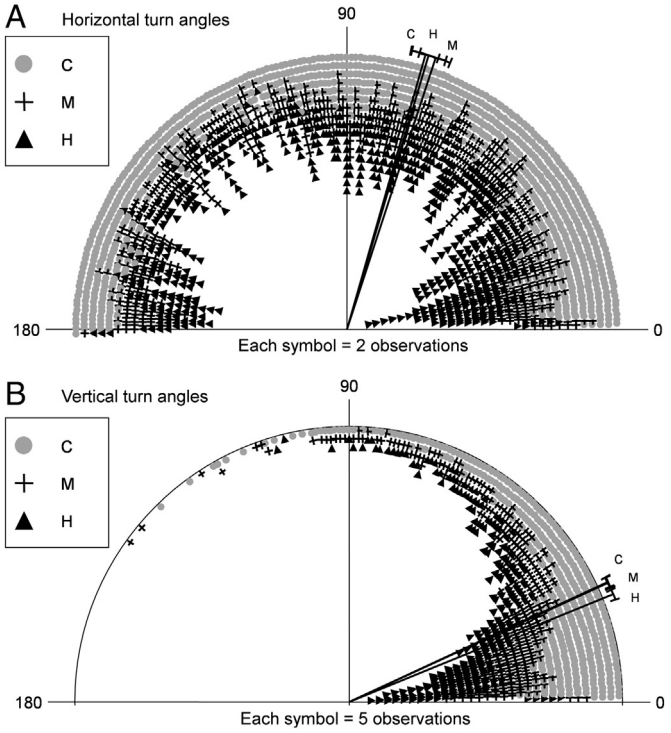

Fig. 2. Turn angles of Atlantic herring (*Clupea harengus* L.) larvae. Horizontal (A) and vertical (B) turn angles of Atlantic herring (*Clupea harengus* L.) larvae at 34-dph for the three pCO_2 treatment levels (C — 370 μatm, M — 1800 μatm, H — 4200 μatm). Black lines from the center denote mean vector angle and arcs at the end of the lines represent 95% confidence interval for each pCO_2 treatment.

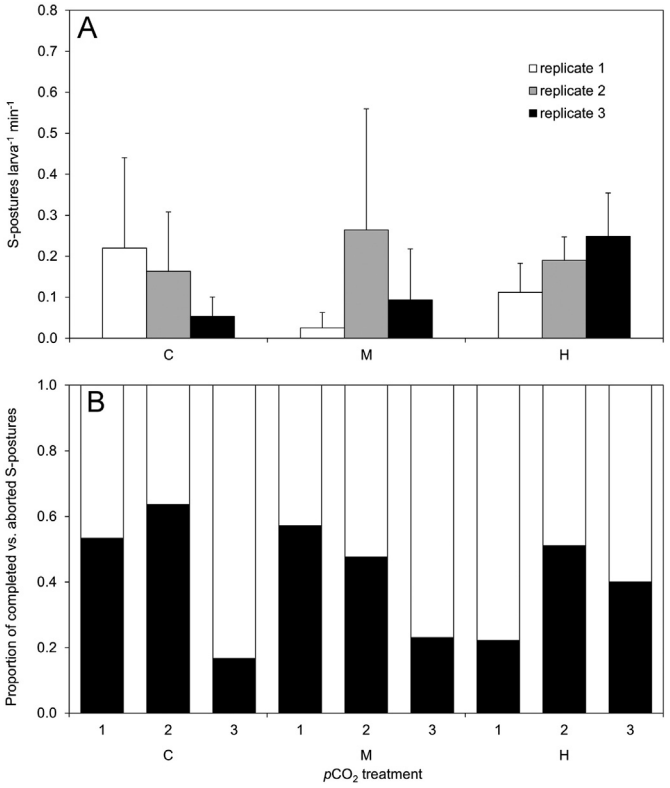

Fig. 3. The frequency of S-postures (A) and the proportion of completed and aborted S-postures (B) in Atlantic herring (*Clupea harengus* L.) larvae at 34-dph for the three pCO_2 treatment levels (C — 370 μatm, M — 1800 μatm, H — 4200 μatm). Panel A: error bars are standard deviations. Panel B: x-axis labels 1, 2 and 3 refer to the 3 replicates per pCO_2 treatment; black and white bars represent completed and aborted S-postures, respectively.

larvae with behavioral choice impairments may still be swimming and feeding normally. Importantly, the earlier studies that reported pCO_2-related behavioral effects in choice experiments did not concomitantly observe swimming or foraging behavior.

Negative effects of high pCO_2 on larval growth and survival have been reported in some marine fishes (e.g. Baumann et al., 2012; Frommel et al., 2012), but not others (e.g. Hurst et al., 2012, 2013; Munday et al., 2011). Further, Miller et al. (2012) reported that the reduced growth and survival observed in juvenile anemonefishes (*Amphiprion melanopus*) reared at high pCO_2 levels were reversed when the parents experienced the same pCO_2 conditions as the juveniles — that is, rapid transgenerational adaptation (Donelson et al., 2012). Larval reef fish exposed to elevated pCO_2 appear to lose their ability to discriminate between ecologically important chemical cues, such as odors from different habitat types, kin and non-kin, and the smell of predators and prey (Cripps et al., 2011; Dixson et al., 2010; Munday et al., 2009). Response to auditory cues also appears to be affected (Simpson et al., 2011) and a range of other behavioral problems have been detected, including the loss of behavioral lateralization (Domenici et al., 2012). Oxygen consumption, swimming duration, activity level and orientation frequency all decreased in dolphinfish (*Coryphaena hippurus*) exposed to elevated pCO_2 (Pimentel et al., 2014).

The preceding paragraphs summarize groupings of studies that arrive at seemingly contradictory conclusions about the effect of ocean acidification on fishes. There are many possible reasons for these inconsistencies: the biological end point measured; the duration and manner in which the pCO_2 treatment was delivered; the age and developmental state of the fish when challenged with higher pCO_2; the condition-nutritional status of the fish; the natural variability (or lack thereof) in pCO_2 in the species'/populations' natural environment; the life history strategy of the species (e.g. *r* vs. *k*); etc.

It also appears that inter-individual variation in the behavioral responses of some fishes to elevated pCO_2 selects for tolerant individuals, possibly resulting in resilience to ocean acidification (Munday et al., 2012). In this context, when measurements are made on individuals that have been exposed to high pCO_2 for some time (34 days in the

Table 2
Effect of different pCO_2 treatments on the horizontal (HTA) and vertical (VTA) swimming turn angles of 34-day post-hatch Atlantic herring (*Clupea harengus* L.) larvae. Results of circular statistics (Mardia–Watson Wheeler tests: MWW) are reported in the table. pCO_2 treatments: C = control, M = medium, H = high, NS = non-significant difference.

Swimming kinematic variable	Treatment	N	Median	Min–max	MWW test	W	p	Conclusion
VTA	C	1813	19°	0–133°	CvM	0.976	0.614	NS
	M	1350	20°	0–143°	CvH	4.737	0.094	NS
	H	1197	17°	0–105°	MvH	5.949	0.051	NS
HTA	C	1757	71°	0–180°	CvM	0.459	0.795	NS
	M	1294	68°	0–180°	CvH	4.78	0.092	NS
	H	1160	69°	0–180°	MvH	1.772	0.412	NS

experiment reported here), one could be observing those individuals that have been selected for their tolerance to pCO_2 — and that might be why there is no effect. Higher within-treatment variability in the swimming kinematics of herring larvae in the elevated pCO_2 treatment indicates the possibility that a mixture of individuals with slightly impaired vs. normal swimming behavior is present in the population. Impaired swimming behavior could be exhibited by larvae experiencing tissue damage. Frommel et al. (2014) reported severe tissue damage in the liver, kidney and pancreas in the larvae from the elevated pCO_2 treatments at 25-dph, which later decreased in proportion by 39-dph. Therefore, we consider it probable that at 34-dph, some surviving larvae in the elevated pCO_2 treatments still had tissue damage, which resulted in higher within-treatment variability. Analysis of the proteomic response of the herring larvae from this experiment at 34-dph showed that protein expression was not significantly affected by elevated pCO_2 (Maneja et al., 2014). Franke and Clemmesen (2011) reported resilience of newly hatched herring larvae to elevated pCO_2 — the RNA/DNA ratio decreased at high pCO_2 levels, but larval length, dry weight, yolk sac area and otolith area were unaffected.

While herring larvae appear to be resilient to ocean acidification on its own, the synergistic effects of multiple stressors (Gao et al., 2012; Pörtner et al., 2005) — continued increases in atmospheric CO_2, increasing seawater temperatures, expansion of hypoxic zones, changes in levels of water turbulence, and decreasing food availability and/or quality — has yet to be assessed.

Acknowledgments

The authors thank the following colleagues who contributed significantly to the conduct of the study: 1) Espegrend mesocosm experiment team — Dr. Mei-Yu Chang, Frank Midtøy, Vibeke Lokøy, Dr. Knut W. Vollset, Oddbjørn Seljeset, Agnes Aadnesen, Tomas Sørlie, Gladys Maneja, and Håkon Otterå. 2) Institute of Marine Research Austevoll team — Dr. Yuichi Fukunishi and Roberto Racca. 3) Water chemistry team — Dr. Richard Bellerby, Hildegunn Almelid, and Jane Strømstad. We also thank two anonymous reviewers for comments that improved the clarity and accuracy of the presentation. Funding support was provided through the European Marie Curie Initial Training Network "Calcification by Marine Organisms" (CalMarO) and the European Community's Seventh Framework Programme (FP7/2007–2013) "European Project on Ocean Acidification" (EPOCA, grant agreement N211384). The study was also supported by the project "Biological Impacts of Ocean ACIDification" (BIOACID), funded by the German Ministry for Education and Research (BMBF), and by the Norwegian Institute of Marine Research ("fine-scale behavioral interactions in the plankton" and "biological effects of ocean acidification" projects to HIB). The experiments were conducted at the Norwegian National Mesocosm Centre, Espegrend, in cooperation with the Department of Biology, University of Bergen and at the Institute of Marine Research's Austevoll Research Station. **[SS]**

References

Bates, N.R., Mathis, J.T., Cooper, L.W., 2009. Ocean acidification and biologically induced seasonality of carbonate mineral saturation states in the western Arctic Ocean. J. Geophys. Res. 114, C11007 (21 pp.).

Batschelet, E., 1981. Circular statistics in biology. Academic Press, New York (371 pp.).

Batty, R.S., 1987. Effect of light intensity on activity and food-searching of larval herring, *Clupea harengus*: a laboratory study. Mar. Biol. 94, 323–327.

Baumann, H., Talmage, S.C., Gobler, C.J., 2012. Reduced early life growth and survival in a fish in direct response to increased carbon dioxide. Nat. Clim. Chang. 2, 38–41.

Beyer, J.E., 1980. Feeding success of clupeoid fish larvae and stochastic thinking. Dana 1, 65–91.

Browman, H.I., O'Brien, W.J., 1992a. The ontogeny of search behavior in the white crappie, *Pomoxis annularis*. Environ. Biol. Fish 34, 181–195.

Browman, H.I., O'Brien, W.J., 1992b. Foraging and prey search behaviour of golden shiner (*Notemigonus crysoleucas*) larvae. Can. J. Fish. Aquat. Sci. 49 (4), 813–819.

Browman, H.I., St-Pierre, J.F., Skiftesvik, A.B., Racca, R.G., 2003. Behavior of Atlantic cod (*Gadus morhua*) larvae: an attempt to link maternal condition with larval quality. In: Browman, H.I., Skiftesvik, A.B. (Eds.), The Big Fish Bang. Proceedings of the 26th Annual Larval Fish Conference. Institute of Marine Research, Bergen, Norway, pp. 71–95.

Checkley Jr., D.M., 1982. Selective feeding by Atlantic herring (*Clupea harengus*) larvae on zooplankton in natural assemblages. Mar. Ecol. Prog. Ser. 9, 245–253.

Cohen, R.E., Lough, R.G., 1983. Prey field of larval herring *Clupea harengus* on a continental shelf spawning area. Mar. Ecol. Prog. Ser. 10, 211–222.

Cripps, I.L., Munday, P.L., McCormick, M.I., 2011. Ocean acidification affects prey detection by a predatory reef fish. PLoS ONE 6 (7), e22736. http://dx.doi.org/10.1371/journal.pone.0022736.

Dixson, D.L., Munday, P.L., Jones, G.P., 2010. Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. Ecol. Lett. 13, 68–75.

Domenici, P., Allan, B., McCormick, M.I., Munday, P.L., 2012. Elevated carbon dioxide affects behavioral lateralization in a coral reef fish. Biol. Lett. 8 (1), 78–81.

Donelson, J.M., Munday, P.L., McCormick, M.I., Pitcher, C.R., 2012. Rapid transgenerational acclimation of a tropical reef fish to climate change. Nat. Clim. Chang. 2, 30–32.

Fabry, V.J., McClintock, J.B., Mathis, J.T., Grebmeier, J.M., 2009. Ocean acidification at high latitudes: the bellwether. Oceanography 22 (4), 160–171.

Ferrari, M.C.O., Manassa, R.P., Dixson, D.L., Munday, P.L., McCormick, M.I., Meekan, M.G., Sih, A., Chivers, D.P., 2012. Effects of ocean acidification on learning in coral reef fishes. PLoS ONE 7 (2), e31478. http://dx.doi.org/10.1371/journal.pone.0031478.

Folkvord, A., Høie, H., Johannessen, A., Solbakken, T., 2009. Effects of prey concentration, light regime, and parental origin on growth and survival of herring larvae under controlled experimental conditions. ICES J. Mar. Sci. 66, 1702–1709.

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

Frommel, A.Y., Maneja, R.H., Lowe, D., Malzahn, A.M., Geffen, A.J., Folkvord, A., Piatkowski, U., Reusch, T., Clemmesen, C., 2012. Severe tissue damage in Atlantic cod larvae under increasing ocean acidification. Nat. Clim. Chang. 2, 42–46.

Frommel, A.Y., Maneja, R., Lowe, D., Pascoe, C.K., Geffen, A.J., Folkvord, A., Piatkowski, U., Clemmesen, C., 2014. Organ damage in Atlantic herring larvae as a result of ocean acidification. Ecol. Appl. 24 (5), 1131–1143.

Fuiman, L.A., 1989. Vulnerability of Atlantic herring larvae to predation by yearling herring. Mar. Ecol. Prog. Ser. 51, 291–299.

Gangstø, R., Gehlen, M., Schneider, B., Bopp, L., Aumont, O., Joos, F., 2008. Modeling the marine aragonite cycle: changes under rising carbon dioxide and its role in shallow water $CaCO_3$ dissolution. Biogeosciences 5, 1057–1072.

Gao, K., Helbling, E.W., Häder, D.-P., Hutchins, D.A., 2012. Responses of marine primary producers to interactions between ocean acidification, solar radiation, and warming. Mar. Ecol. Prog. Ser. 470, 167–180.

Geffen, A.J., 2009. Advances in herring biology: from simple to complex, coping with plasticity and adaptability. ICES J. Mar. Sci. 66, 1688–1695.

Hamilton, T.J., Holcombe, A., Tresguerres, M., 2014. CO_2-induced ocean acidification increases anxiety in Rockfish via alteration of GABA-A receptor functioning. Proc. R. Soc. B 281. http://dx.doi.org/10.1098/rspb.2013.2509 (20132509).

Haslob, H., Rohlf, N., Schnack, D., 2009. Small scale distribution patterns and vertical migration of North Sea herring larvae (*Clupea harengus*, Teleostei: Clupeidea) in relation to abiotic and biotic factors. In: Clemmesen, C., Malzahn, A.M., Peck, M.A., Schnack, D. (Eds.), Advances in early life history study of fish. Scientia Marina 73S1. http://dx.doi.org/10.3989/scimar.2009.73s1013 (October 13–22, 2009. ISSN: 0214–8358).

Hauss, H.M., Peck, M.A., 2009. Comparing observed and modelled growth of larval herring (*Clupea harengus*): testing individual-based model parameterisations. In: Clemmesen, C., Malzahn, A.M., Peck, M.A., Schnack, D. (Eds.), Advances in early life history study of fish. Scientia Marina 73S1. http://dx.doi.org/10.3989/scimar.2009.73s1037 (October 13–22, 2009. ISSN: 0214–8358).

Houde, E.D., 2002. Mortality. In: Fuiman, L.A., Werner, R.G. (Eds.), Fishery Science: The Unique Contributions of Early Life Stages. Blackwell Science, Oxford, pp. 64–87.

Hunter, J.R., 1972. Swimming and feeding behavior of larval anchovy (*Engraulis mordax*). U.S. Fish. Bull. 70 (3), 821–838.

Hurst, T.P., Fernandez, E.R., Mathis, J.T., Miller, J.A., Stinson, C.M., Ahgeak, E.F., 2012. Resiliency of juvenile walleye pollock to projected levels of ocean acidification. Aquat. Biol. 17, 247–259.

Hurst, T.P., Fernandez, E.R., Mathis, J.T., 2013. Effects of ocean acidification on hatch size and larval growth of walleye pollock (*Theragra chalcogramma*). ICES J. Mar. Sci. 70 (4), 812–822.

IPCC, 2007. Climate change 2007: the physical science basis. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., et al. (Eds.), Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA (996 pp.).

Jutfelt, F., de Souza, K.B., Vuylsteke, A., Sturve, J., 2013. Behavioural disturbances in a temperate fish exposed to sustained high-CO_2 levels. PLoS ONE 8 (6), e65825. http://dx.doi.org/10.1371/journal.pone.0065825.

MacKenzie, B.R., Kiørboe, T., 1995. Encounter rates and swimming behavior of pause-travel and cruise larval fish predators in calm and turbulent laboratory environments. Limnol. Oceanogr. 40 (7), 1278–1289.

Maneja, R.H., Frommel, A.Y., Browman, H.I., Clemmesen, C., Geffen, A.J., Folkvord, A., Piatkowski, U., Durif, C.M.F., Bjelland, R., Skiftesvik, A.B., 2013. The swimming kinematics of larval Atlantic cod, *Gadus morhua* L., are resilient to elevated seawater pCO_2. Mar. Biol. 160, 1963–1972.

Maneja, R.H., Dineshram, R., Thiyagarajan, V., Skiftesvik, A.B., Frommel, A.Y., Clemmesen, C., Geffen, A.J., Browman, H.I., 2014. The proteome of Atlantic herring (Clupea harengus L.) larvae is resistant to elevated pCO_2. Mar. Pollut. Bull 86 (1–2), 154–160.

Miller, G.M., Watson, S.-A., Donelson, J.M., McCormick, M.I., Munday, P.L., 2012. Parental environment mediates impacts of increased carbon dioxide on a coral reef fish. Nat. Clim. Chang. 2, 858–861.

Munday, P.L., Dixson, D.L., Donelson, J.M., Jones, G.P., Pratchett, M.S., Devitsina, G.V., Døving, K.B., 2009. Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. Proc. Natl. Acad. Sci. U. S. A. 106 (6), 1848–1852.

Munday, P.L., Dixson, D.L., McCormick, M.I., Meekan, M., Ferrari, M.C.O., Chivers, D.P., 2010. Replenishment of fish populations is threatened by ocean acidification. Proc. Natl. Acad. Sci. U. S. A. 107 (29), 12930–12934.

Munday, P.L., Gagliano, M., Donelson, J.M., Dixson, D.L., Thorrold, S.R., 2011. Ocean acidification does not affect the early life history development of a tropical marine fish. Mar. Ecol. Prog. Ser. 423, 211–221.

Munday, P.L., McCormick, M.I., Meekan, M., Dixson, D.L., Watson, S.-A., Chivers, D.P., Ferrari, M.C.O., 2012. Selective mortality associated with variation in CO_2 tolerance in a marine fish. Ocean Acidification 1, 1–5.

Munk, P., Kiørboe, T., 1985. Feeding behavior and swimming activity of larval herring (*Clupea harengus*) in relation to density of copepod nauplii. Mar. Ecol. Prog. Ser. 24, 15–21.

Nash, R.D.M., Dickey-Collas, M., Kell, L.T., 2009. Stock and recruitment in North Sea herring (*Clupea harengus*); compensation and depensation in the population dynamics. Fish. Res. 95 (1), 88–97.

Nilsson, G.E., Dixson, D.L., Domenici, P., McCormick, M.I., Sørensen, C., Watson, S.-A., Munday, P.L., 2012. Near-future carbon dioxide levels alter fish behavior by interfering with neurotransmitter function. Nat. Clim. Chang. 2, 201–204.

Olafsson, J., Olafsdottir, S.R., Benoit-Cattin, A., Danielsen, M., Arnarson, T.S., Takahashi, T., 2009. Rate of Iceland Sea acidification from time series measurements. Biogeosci. Discuss. 6, 5251–5270.

Payne, M.R., Hatfield, E.M.C., Dickey-Collas, M., Falkenhaug, T., Gallego, A., Gröger, J., Licandro, P., Llope, M., Munk, P., Röckmann, C., Schmidt, J.O., Nash, R.D.M., 2009. Recruitment in a changing environment: the 2000s North Sea herring recruitment failure. ICES J. Mar. Sci. 66 (2), 272–277.

Peck, M.A., Huebert, K.B., Llopiz, J.K., 2012a. Intrinsic and extrinsic factors driving match–mismatch dynamics during the early life history of marine fishes. In: Woodward, G., Jacob, U., O'Gorman, E.J. (Eds.), Advances in Ecological Research 47, pp. 177–302.

Peck, M.A., Kanstinger, P., Holste, L., Martin, M., 2012b. Thermal windows supporting survival of the earliest life stages of Baltic herring (*Clupea harengus*). ICES J. Mar. Sci. 69 (4), 529–536.

Pimentel, M., Pegado, M., Repolho, T., Rosa, R., 2014. Impact of ocean acidification in the metabolism and swimming behavior of the dolphinfish (*Coryphaena hippurus*) early larvae. Mar. Biol. 161, 725–729.

Pörtner, H.-O., Langenbuch, M., Michaelidis, B., 2005. Synergistic effects of temperature extremes, hypoxia, and increases in CO_2 on marine animals: from earth history to global change. J. Geophys. Res. 110, C09S10. http://dx.doi.org/10.1029/2004JC002561.

Rosenthal, H., 1968. Schwimmverhalten und Schwimmgeschwindigkeit bei den Larven des Herings Clupea harengus. Helgoländer Wiss. Meeresun. 18, 453–486.

Rosenthal, H., Hempel, G., 1970. Experimental studies in feeding and food requirements of herring larvae (*Clupea harengus* L.). In: Steele, J.H. (Ed.), Symp. Marine Food Chains. Univ. of Aarhus, Denmark 1968. Oliver and Boyd, Edinburg, pp. 344–364.

Seljeset, O., Vollset, K., Folkvord, A., Geffen, A.J., 2010. The role of prey concentration and size range in the growth and survival of larval cod. Mar. Biol. Res. 6, 251–262.

Simpson, S.D., Munday, P.L., Wittenrich, M.L., Manassa, R., Dixson, D.L., Gagliano, M., Yan, H.Y., 2011. Ocean acidification erodes crucial auditory behavior in a marine fish. Biol. Lett. 7 (6), 917–920.

Steinacher, M., Joos, F., Frölicher, T.L., Plattner, G.K., Doney, S.C., 2009. Imminent ocean acidification in the Arctic projected with the NCAR global coupled carbon cycle-climate model. Biogeosciences 6, 515–533.

Thomsen, J., Gutowska, M.A., Saphörster, J., Heinemann, A., Trübenbach, K., Fietzke, J., Hiebenthal, C., Eisenhauer, A., Körtzinger, A., Wahl, M., Melzner, F., 2010. Calcifying invertebrates succeed in a naturally CO_2-rich coastal habitat but are threatened by high 7 levels of future acidification. Biogeosciences 7, 3879–3891.

Videler, J.J., 1993. Fish swimming. Fish and Fisheries Series 10. Chapman and Hall, London (269 pp.).

Vollset, K.W., Folkvord, A., Browman, H.I., 2011. Foraging behavior of larval cod (*Gadus morhua*) at low light intensities. Mar. Biol. 158, 1125–1133.