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The swimming kinematics of larval Atlantic cod, *Gadus morhua* L., are resilient to elevated seawater pCO_2

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Abstract Kinematics of swimming behavior of larval Atlantic cod, aged 12 and 27 days post-hatch (dph) and cultured under three pCO_2 conditions (control-370, medium-1800, and high-4200 µatm) from March to May 2010, were extracted from swim path recordings obtained using silhouette video photography. The swim paths were analyzed for swim duration, distance and speed, stop duration, and horizontal and vertical turn angles to determine whether elevated seawater pCO_2—at beyond near-future ocean acidification levels—affects the swimming kinematics of Atlantic cod larvae. There were no significant differences in most of the variables tested: the swimming kinematics of Atlantic cod larvae at 12 and 27 dph were highly resilient to extremely elevated pCO_2 levels. Nonetheless, cod larvae cultured at the highest pCO_2 concentration displayed vertical turn angles that were more restricted (median turn angle, 15°) than larvae in the control (19°) and medium (19°) treatments at 12 dph (but not at 27 dph). Significant reduction in the stop duration of cod larvae from the high treatment (median stop duration, 0.28 s) was also observed compared to the larvae from the control group (0.32 s) at 27 dph (but not at 12 dph). The functional and ecological significance of these subtle differences are unclear and, therefore, require further investigation in order to determine whether they are ecologically relevant or spurious.

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Introduction

The high-latitude regions, including the Norwegian Sea and Barents Sea, are referred to as the "bellwether" for future trends of ocean acidification (Fabry et al. 2009). These areas are sensitive to ocean acidification because of increased CO_2 solubility at low water temperatures, greater air-sea gas exchange as sea ice loss increases from global warming, and CO_2 remineralization of high organic carbon load from seasonal primary production and low alkalinity riverine inputs. According to the global coupled carbon cycle climate model of Steinacher et al. (2009), the Arctic surface waters will experience the largest model simulated pH changes over the twenty-first century with hydrogen ion concentration increases of up to 185 % or an equivalent pH decline of 0.45 units in response to a global mean atmospheric CO_2 concentration of 850 ppm under the A2 IPCC business-as-usual scenario (IPCC 2007). Assuming that the ocean and atmosphere are in equilibrium with respect to CO_2, partial pressure of CO_2 (pCO_2) in seawater roughly mimics that of the atmosphere (Gattuso and Lavigne 2009). Further, localized decreases in pH—in addition to these global predictions—can result from seasonal upwelling of high pCO_2 bottom water. Such events were recorded inside the bay and along the inner shelf of the Gulf of Alaska in September 2008 with aragonite under-saturated waters measured close to the surface until about 450 m deep (Fabry et al. 2009). Seasonal occurrences of aragonite-undersaturated subsurface waters, resulting from anthropogenic CO_2 uptake,

were reported in 2002 to 2004 in the Chukchi Sea—which, like the Barents Sea, functions as an inflow shelf to the Arctic Ocean (Bates et al. 2009). The Norwegian Sea and Barents Sea act as a carbon sink from the first part of the year until the end of summer due to the biological draw-down of pCO_2 and cooling of surface water during transit (Findlay et al. 2008; Bates and Mathis 2009). Gislefoss et al. (1998) reported typical surface water pCO_2 values in the Norwegian Sea during winter at 357 µatm and summer as low as 270–300 µatm from 1991 to 1994. Thus, pelagic organisms in the Norwegian and Barents Sea might be naturally adapted to lower pCO_2 and high aragonite saturation during the summer period. However, how future ocean acidification events may impact these organisms is an open question.

A number of studies have reported on the detrimental effects of ocean acidification on the behavior and sensory responses of marine fishes. White seabass larvae grown at 2,500 µatm were lethargic and swam less than control larvae (Huelsenbeck 2010). Damage to the olfactory ability of a clownfish, *Amphiprion percula*, resulted in a loss of homing ability to reef habitat and suitable settlement sites at pH 7.8 and 7.6 (Munday et al. 2009b) and loss of predator detection/avoidance abilities at 700–1,000 µatm CO_2 (Munday et al. 2010; Dixson et al. 2010). The brown dottyback, *Pseudochromis fuscus*, exposed to elevated pCO_2 exhibited a negative reaction to the smell of injured prey seemingly making them less able to respond to changing food availability (Cripps et al. 2011). Hearing ability of juvenile clownfish reared in elevated pCO_2 of 600–900 µatm was also damaged with juveniles attracted to reef noise that exposed them to greater predation risks (Simpson et al. 2011). Domenici et al. (2012) provided evidence of damage to brain function resulting from elevated pCO_2 by showing a reduced level of lateralization or ability to decide between left and right turns in a reef fish, *Neopomacentrus azysron*. Nilsson et al. (2012) reported that elevated pCO_2 affects neurotransmitter function (GABA-A receptors) in the brain of larval reef fish species resulting in seemingly maladaptive olfactory choices and loss of behavioral lateralization. The ability of damselfish, *Pomacentrus amboinensis,* to learn from chemical and visual information of a predator was also damaged at elevated pCO_2, i.e. non-display of anti-predatory response even with previous exposure to predator cues (Ferrari et al. 2012).

Changes in the swimming behavior of fish larvae—in response to ocean acidification, for example—are difficult to examine due to the very small size and transparency of the fish. Fish kinematics analyzes changes in position as a function of time without reference to hydrodynamic forces (Videler 1993). Fish kinematics has wide application in foraging studies such as the characterization of prey search behavior (O'Brien et al. 1989; Browman and O'Brien 1992), foraging under variable prey concentrations (Coughlin et al. 1992; Puvanendran et al. 2002) and under different spectral qualities and intensities of light (Browman et al. 1994; Vollset et al. 2011), and in escape responses from predators (Webb 1981; Domenici 2001; Skajaa and Browman 2007). Given all of the behavioral changes observed in the studies cited above, it seems reasonable to expect that elevated pCO_2 will also have an effect on the kinematics of larval movements.

One of the most economically important commercial fish species in the north Atlantic is the Atlantic cod, *Gadus morhua* L. The largest Atlantic cod stock is the Arcto-Norwegian cod in the Barents Sea (Suthers and Sundby 1993). Arcto-Norwegian cod and Norwegian coastal cod release pelagic eggs along a 1,200-km coastline from mid- to northern Norway. Eggs hatch and are advected to the Barents Sea during an approximately 100-day planktonic larval and early juvenile phase (Houde and Zastrow 1993; Suthers and Sundby 1993). The timing of release of the pelagic early life stages of these two cod stocks coincides with the seasonal surface increase in calcite and aragonite saturation states and with the draw-down of pCO_2 during spring and summer primary production (Findlay et al. 2008). This decreases the likelihood that early life stages of cod in this region are pre-adapted to fluctuating low pH.

Considering the economic importance of Atlantic cod, and its presence at high latitudes in waters that are sensitive to ocean acidification, this study was conducted to investigate whether elevated seawater pCO_2 affects the swimming kinematics of Atlantic cod larvae. pCO_2 values beyond near-future ocean acidification scenarios were selected to assess the possible effect of extreme acidification events such as those associated with upwelling.

Materials and methods

Seawater manipulation

Cod larvae were grown in land-based mesocosms at the University of Bergen's Espegrend Marine Station from March to May 2010 at three pCO_2 levels: control (370 µatm), medium (1,800 µatm), and high (4,200 µatm), with three replicates for each treatment level (Table 1).

The 2650-L replicate tanks of 1.5 m height were placed inside two water baths to keep the temperature relatively stable with a starting temperature of 5 °C in March which rose to 10 °C by May. Seawater was supplied to the tanks using a flow through system from a 40-m-deep water intake. Salinity was relatively constant at a mean of 33.3 psu, and dissolved oxygen concentration remained above 90 % saturation. For further details, see Frommel et al. (2012).

Table 1 Carbonate system variables measured weekly for eight weeks for the ocean acidification experiment with larval Atlantic cod (*Gadus morhua* L.)

Treatment	Replicate	Temperature (°C)	C_T (μmol kg^{-1} SW)	A_T (μmol kg^{-1} SW)	pCO_2 (μatm)	pH_T
Control, C	1	7.2 ± 0.9	2103 ± 39	2271 ± 41	368 ± 78	8.08 ± 0.09
	2	7.2 ± 0.8	2107 ± 35	2283 ± 36	373 ± 83	8.07 ± 0.09
	3	7.2 ± 0.8	2110 ± 36	2291 ± 27	359 ± 70	8.09 ± 0.07
Medium, M	1	7.1 ± 0.8	2340 ± 40	2296 ± 23	1866 ± 559	7.43 ± 0.12
	2	7.2 ± 0.9	2319 ± 37	2283 ± 37	1810 ± 342	7.44 ± 0.09
	3	7.2 ± 0.8	2332 ± 77	2286 ± 46	1957 ± 1019	7.46 ± 0.22
High, H	1	7.1 ± 0.8	2461 ± 35	2284 ± 34	4170 ± 861	7.08 ± 0.10
	2	7.1 ± 0.7	2490 ± 49	2293 ± 30	4547 ± 872	7.05 ± 0.08
	3	7.2 ± 0.7	2464 ± 60	2296 ± 30	4026 ± 898	7.10 ± 0.10

Mean values with standard deviation: C_T total dissolved inorganic carbon, A_T total alkalinity, pCO_2 partial pressure of CO_2, pH_T (total scale) at a salinity of 33.3

The targeted pCO_2 levels were achieved by bubbling CO_2 through diffusers to the bottom of the tanks close to the water inflow and aeration bubbles. This provided rapid CO_2 mixing in the water column and appropriate water circulation. The amount of CO_2 added to the tanks was regulated by magnetic valves using a feedback mechanism from a pH probe in each tank, which was connected to an Aquastar IKS computer. The pH probes provided continuous daily pH_{NBS} measurements of all tanks at 15-min intervals, which were recorded by the computer. In addition, daily measurements of pH in the tanks were made with a hand-held laboratory glass pH probe (WTW) to check the pH values of the Aquastar IKS. The WTW pH probe was calibrated with seawater standard and seawater-certified reference material (Oceanic Carbon Dioxide Quality Control, Dr. Andrew G. Dickson, Scripps Institution of Oceanography). Water samples for dissolved inorganic carbon (DIC) and alkalinity were also collected weekly for the calculation of the total carbonate chemistry using CO2SYS (Lewis and Wallace 1998).

Larval rearing

Newly fertilized Norwegian coastal cod eggs were obtained from the Parisvatnet Field Station of the Institute of Marine Research on March 25, 2010. The eggs were immediately transferred to incubation buckets floating inside each replicate tank at the Espegrend mesocosm facility at an equal stocking density. After 3 days, the pH was adjusted to the treatment level. Dead eggs were removed from the incubation buckets until the 50 % hatching day on April 9, 2010, also designated as 0 days post-hatch (dph). On the same day, the newly hatched cod larvae were redistributed among the replicate tanks so that each treatment had a similar initial stocking density (ca. 4 larvae L^{-1}).

The larvae were fed with natural zooplankton. Seawater near the station was filtered daily using a Hydrotech size-selective filter system (Seljeset et al. 2010). Zooplankton of 80–250 μm (primarily copepod nauplii and rotifers) were added daily to each tank initially, and replaced gradually by zooplankton 350–500 μm (nauplii and small copepods) by 34 dph. The residual zooplankton density in each tank was monitored daily prior to feeding to achieve a prey density of 2,000 prey L^{-1} (following Puvanendran et al. 2002).

Since the rearing experiment was performed outdoors, the larvae were exposed to a natural light cycle and intensity. Average light intensities measured using a LI-COR Underwater Quantum Sensor during a sunny day and zero cloud cover were 330 and 93.6 μmol s^{-1} m^{-2} at 5-cm below water surface and at bottom of tanks, respectively.

Handling of animals in the mesocosm experiment was conducted using the animal experimentation permit ID2346 granted by the Animal Welfare Committee as determined under the Norwegian Animal Welfare Act.

Video recording of larval swimming behavior

The swimming behavior of 12- and 27-dph Atlantic cod larvae was recorded using silhouette video photography (SVP). The use of SVP to analyze the kinematics of behavior of cod larvae is a powerful technique compared to conventional video imaging tools because it allows for recording of behavior with a large depth and field, very good resolution, and low intensity light sources to achieve the silhouette effect (see Browman et al. 2003).

During the night prior to observation and recording, around 60 larvae were randomly sampled from each replicate tank and placed in floating buckets in tanks with mesh bottoms that kept food out. Around 4 am, the sampled larvae were transferred to cylindrical transparent plastic bags with 7 liters of seawater from each tank.

The plastic bags were then placed inside cooler boxes to keep water temperature stable during transport. Transport of larvae from the Espegrend mesocosm facility to the Institute of Marine Research's Austevoll Research Station took about 2 h. Transport of cod larvae to the Austevoll Research Station was previously reported to have no detrimental effect on the larvae (Vollset et al. 2011). The larvae were kept in the dark from the time of sampling until 10 min prior to recording to inhibit feeding. Thus, it is assumed that during recording, the larvae were hungry and motivated to look for prey.

Approximately 50 larvae from each replicate tank were transferred into separate $20 \times 20 \times 20$ cm observation tanks inside the SVP room (in darkness). One observation tank was used for each replicate tank. Seawater from the treatment tanks was used to transport the larvae to the observation tank. The observation tanks were sealed with plastic to reduce efflux of CO_2. A separate test of CO_2 efflux showed that the pH and pCO_2 levels of the three treatments remained distinguishable after simulated transport conditions, although there was a slight loss of approximately 360 μatm from the highest treatment. Room temperature was maintained close to the average mesocosm seawater temperature: 6.7 °C for 12 dph and 8.9 °C for 27 dph. 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. The outer five cm of the observation tanks was covered with black plastic contact paper, which restricted the field of view to the central 15 cm volume of water. This ensured that surface or edge effects did not affect the observations. A known amount of fresh zooplankton was added to the observation tank 10 min prior to recording to motivate foraging behavior. The prey density during the observations was about 550 prey L^{-1}. The total recording time for each replicate was 30 min with temporal resolution of 25 frames per second. Complete descriptions of the observation procedures and the principles of the SVP and the analysis of swim paths are reported in Browman et al. (2003).

Analysis of swim paths

Reconstruction of the individual fish swim paths was done by combining the two orthogonally recorded videos of each observation tank using the TrakFish software (Racca Scientific Consulting and JASCO Research Ltd., Victoria, British Columbia, Canada). In TrakFish, the two videos 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 fish location was derived. Swim paths were reconstructed frame-by-frame from the initial, middle, and last 5 min of the observation to provide representative sampling.

Using the Anapaths software (also from Racca Scientific Consulting and JASCO Research Ltd., Virginia, British Columbia, Canada), paths that were closely adjacent to each other and seemed to resemble 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 namely move duration, distance and speed, stop duration, and horizontal and vertical turn angles (i.e., change in direction after a stop in a horizontal and vertical planes) were extracted using the Anapaths software. Every data point from each reconstructed fish swim path was used as an observation value for statistical analysis. The output files for each replicate tank consisted of a list of recorded—for example—turn angles, which did not differentiate individual fish in the tank.

Data analysis

The horizontal and vertical turn angle components of the swim paths were analyzed using circular statistics (Batschelet 1981). A nonparametric Mardia–Watson–Wheeler test (MWW test) was used to compare distributions of the turn angles between replicates within each treatment, and then between treatments provided that replicates did not show any significant differences. If data showed higher variability between replicates than between treatments, it was evident that treatments had no effect.

For the other variables (stop duration, move duration, move length, and move speed), a nonparametric two-sample Kolmogorov–Smirnov (KS) test was used to compare the distribution of replicates within treatments and then between treatments, again, provided that replicates did not show any significant differences. When a significant difference was found, a Mann–Whitney test was applied to compare the medians between treatments. A 5 % significance level was used in all tests.

Results

Age-related differences

Age-related increase in horizontal turn angle was detected (MWW, $p < 0.001$). Median turn angles for 12- and 27-dph larvae were, respectively, 33° and 38°. Move duration was statistically different between ages (Mann–Whitney test, $p < 0.05$). Older larvae had a higher count of short move durations (25 % percentile was slightly higher in 27-dph larvae).

Turn angles

Turn angles were highly variable between replicates for both age groups, and confidence intervals were generally overlapping (Figs. 1, 2). Only the vertical turn angle at 12 dph had all treatments with homogenous replicates, and a statistically significant difference was found between the high treatment (median turn angle, 15°) and the medium or control treatments (both at 19°) (MWW test $p_{C-H} = 0.006$; $p_{M-H} < 0.001$) (Fig. 1a; Table 2). Vertical turn angles for high pCO_2-treated fish (4,200 µatm) were 21 % narrower than for control and medium cod larvae (Fig. 1a). There was no significant difference between medium and control treatments ($p > 0.05$).

Linear variables The only difference in treatments that was statistically significant, both in distribution and in median, was in 27-dph fish. Individuals in the high treatment showed significantly shorter stop durations than fish in the control treatment (KS test: $p < 0.001$; Mann–Whitney U test: $p < 0.001$) (Fig. 3b; Table 2). This represents a 12.5 % decrease in stop duration relative to the control group. Assuming a 7-h daily "active feeding" period, the median stop durations of the control (0.32 s) and high (0.28 s) treatments were translated into 421.1 and 419.4 min of total daily search time for each treatment, respectively. Since cod larvae are saltatory searchers—that is, they search for prey only while stationary (Galbraith et al. 2004; Hunt von Herbing et al. 2001; Ruzicka and Gallager 2006)—the total daily search time was calculated based on the total number of stops in a given 7-h period and the median duration of the stops for each treatment. Thus, a difference in total daily search time between the control and high treatments was estimated to be 100.8 s.

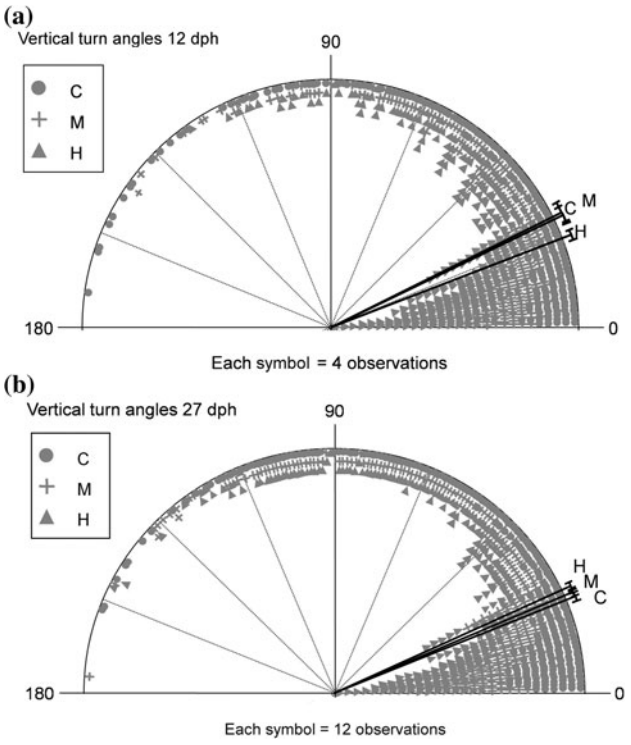

Fig. 1 *Vertical turn angles* of **a** 12-dph and **b** 27-dph Atlantic cod larvae (*Gadus morhua* L.) from three pCO_2 levels (C-380 µatm, M-1800 µatm, H-4200 µatm). *Black lines* from the center denote mean vector angle and *arcs* at the end of the *lines* represent 95 % confidence interval for each pCO_2 treatment

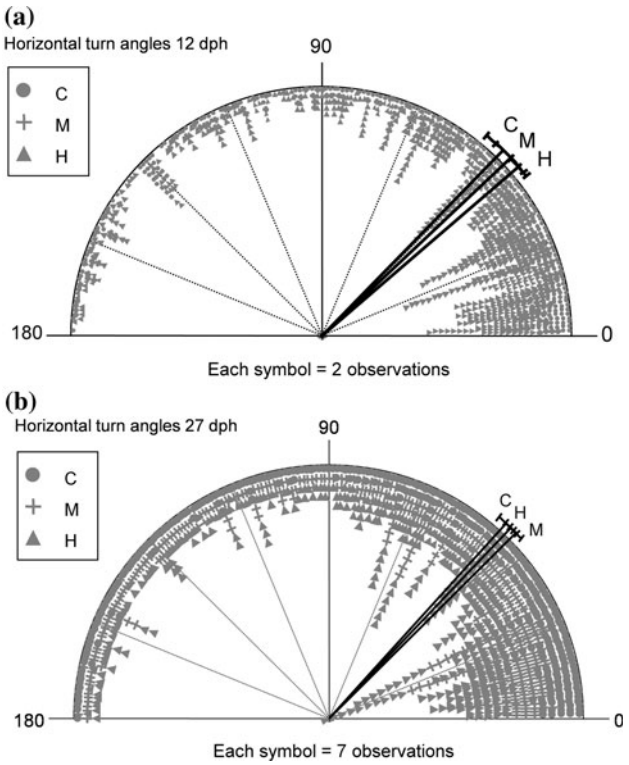

Fig. 2 Horizontal turn angles of **a** 12-dph and **b** 27-dph Atlantic cod larvae (*Gadus morhua* L.) from three pCO_2 levels (C-380 µatm, M-1800 µatm, H-4200 µatm). *Black lines* from the center denote mean vector angle and *arcs* at the end of the *lines* represent 95 % confidence interval for each pCO_2 treatment

Stop and move durations (median values, respectively, 0.28 and 0.32 s) at 12 dph did not vary between control and high treatments (KS test: $p > 0.05$) (Figs. 3a, 4a; Table 2). At 27 dph, pairwise comparison of medium treatment (median move duration, 0.32 s; speed, 5.9 mm s^{-1}) vs. high treatment (median, 0.36 s) for move duration (Fig. 4b) and vs. the control group for move speed (median, 6.0 mm s^{-1}) (Fig. 5b) did not reveal significant differences (KS test: $p > 0.05$).

Treatment comparisons were not carried out for move speed at 12 dph and move distance in both age groups (Fig. 5a, 6; Table 2). In such cases, at least two treatments had high variability among replicates.

Table 2 Summary of statistics

Swim kinematics	Age (dph)	Treatment	*N*	Median	Min–max	Statistical significance: between replicates	Statistical significance between treatments
VTA	12	C	618	19°	0–171°	NS	C ≠ H
		M	673	19°	0–145°	NS	M ≠ H
		H	1323	15°	0–126°	NS	C=M
	27	C	2268	15°	0–159°	*	NT
		M	2595	16°	0–175°	*	
		H	2589	18°	0–154°	NS	
HTA	12	C	350	36°	0–179°	NS	NT
		M	408	34°	0–174°	*	
		H	1000	31°	0–177°	*	
	27	C	1742	40°	0–180°	*	NT
		M	2165	37°	0–180°	*	
		H	2185	38°	0–179°	NS	
SD	12	C	704	0.28	0.1–3.3	NS	C=H
		M	782	0.32	0.1–5.5	*	
		H	1473	0.28	0.1–3.0	NS	
	27	C	2572	0.32	0.1–7.6	NS	C ≠ H
		M	2837	0.28	0.1–3.6	*	
		H	2821	0.28	0.1–3.2	NS	
MD	12	C	763	0.32	0.1–3.4	NS	C=H
		M	842	0.28	0.1–3.5	*	
		H	1583	0.32	0.1–5.1	NS	
	27	C	2700	0.32	0.1–3.2	*	M=H
		M	3012	0.32	0.1–4.2	NS	
		H	2978	0.36	0.1–2.8	NS	
MS	12	C	753	5.9	2.5–20	*	NT
		M	828	6.0	3.2–20	*	
		H	1565	5.9	2.7–20	*	
	27	C	2671	6.0	2.6–20	NS	C=M
		M	2997	5.9	2.6–19	NS	
		H	2958	6.0	2.5–20	*	
ML	12	C	755	2.0	0.30–19	*	NT
		M	831	1.8	0.36–19	*	
		H	1573	2.1	0.36–19	NS	
	27	C	2697	1.9	0.30–20	*	NT
		M	3002	2.2	0.32–20	NS	
		H	2970	2.2	0.36–20	*	

* Significant difference

NS non-significant difference, *NT* no tests were performed because replicates were significantly different within treatments

Nonparametric tests were as follows: Mardia–Watson–Wheeler test (MWW test) for turn angles; Kolmogorov–Smirnov test; and Mann–Whitney test for linear parameters

Discussion

There were clear between-age differences in horizontal turn angle and move duration. However, only cod larvae from the high pCO_2 group showed significant changes in swimming kinematics relative to the control group. Larvae from the high pCO_2 treatment exhibited smaller vertical displacements at 12 dph and shorter stop durations at 27 dph.

Although the functional significance of the statistically significant differences in vertical displacement and stop duration is unclear, it is instructive to examine these changes in the context of existing explanatory frameworks for the mechanisms that modulate repositioning movements of zooplanktivorous fishes. Cod larvae are characterized as saltatory searchers (Hunt von Herbing et al. 2001; Galbraith et al. 2004; Ruzicka 2004). O'Brien et al. (1989) coined this term to describe a stop and go swim pattern and demonstrated that the fish searched for prey only during the brief stationary periods. The relative durations of repositioning movements and pauses are modulated by environmental conditions such as light level

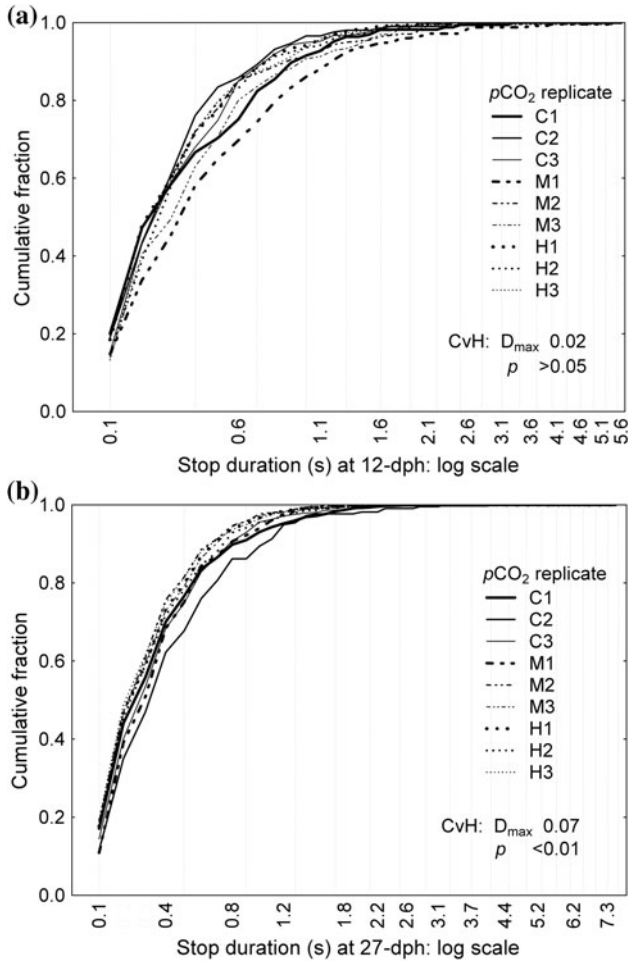

Fig. 3 Cumulative fraction plots for the Kolmogorov–Smirnov tests of stop duration of Atlantic cod larvae (*Gadus morhua* L.) at **a** 12 dph and **b** 27 dph under three pCO_2 levels (C = 370 µatm, M = 1,800 µatm, and H = 4,200 µatm). Within-treatment variability in the M-group prevented comparison with other treatments

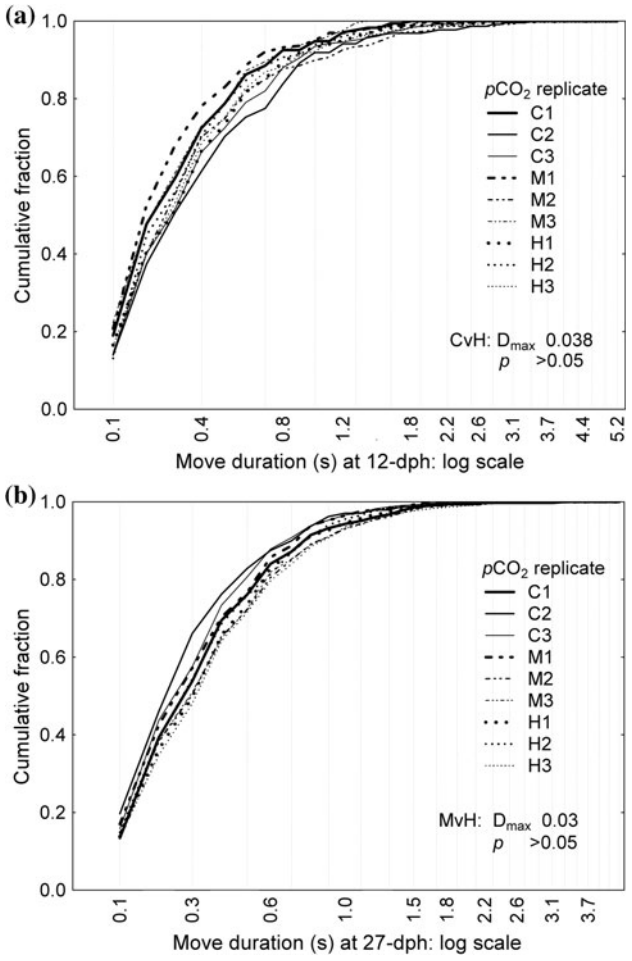

Fig. 4 Cumulative fraction plots for the Kolmogorov–Smirnov tests of move duration of Atlantic cod larvae (*Gadus morhua* L.) at **a** 12 and **b** 27 dph under three pCO_2 levels (C = 370 µatm, M = 1,800 µatm, and H = 4,200 µatm). Within-treatment variability in the M- and C-groups at 12 and 27 dph, respectively, prevented comparison with other treatments

and prey size and/or abundance. Modulation of these kinematic variables results in larvae increasing prey encounter rate when food abundance is low (or the prey are very small) and maintains position within a small area when food is abundant (area-restricted search; see Coughlin et al. 1992). It also has significant implications for energy conservation because foraging activity accounts for up to 80 % of routine metabolism in cod larvae (Ruzicka 2004). The reduction in stop duration (i.e., the prey location phase) at 27 dph could mean that the larvae were spending less time looking for food. We calculated the median stop durations in this study (control = 0.32 s and high = 0.28 s) and comparing to other studies they were relatively shorter than those reported earlier for cod larvae: Ruzicka (2004), 0.78 s for 27 dph and Vollset et al. (2011), 1.64 s for 26-dph larvae observed at dusk. On the other hand, the mean stop durations reported here were closer to those obtained for 6-dph cod larvae, which had 0.3-s mean stop duration (Browman et al. 2003). Roughly estimating, the difference in the median stop durations between the two treatments corresponds to a reduction of 100.8 s in total daily search time.

Skiftesvik et al. (2003) reported significant differences on the vertical turn angles of 5-dph cod larvae feeding on rotifers only versus larvae feeding on rotifers with algae. Larvae feeding on rotifers and algae had greater mean vertical repositioning angles. Browman et al. (2003) also reported an effect of maternal condition on the vertical repositioning angles of 6-dph cod larvae, with wider angles in larvae from well-fed mothers. Although the implications of these observations were not discussed, it is possible that, in both cases, the high concentration and contrast of prey at the edges of the search volume caused the larvae to modulate their movements by turning wider horizontally and vertically. For a wedge-shaped search volume with only a

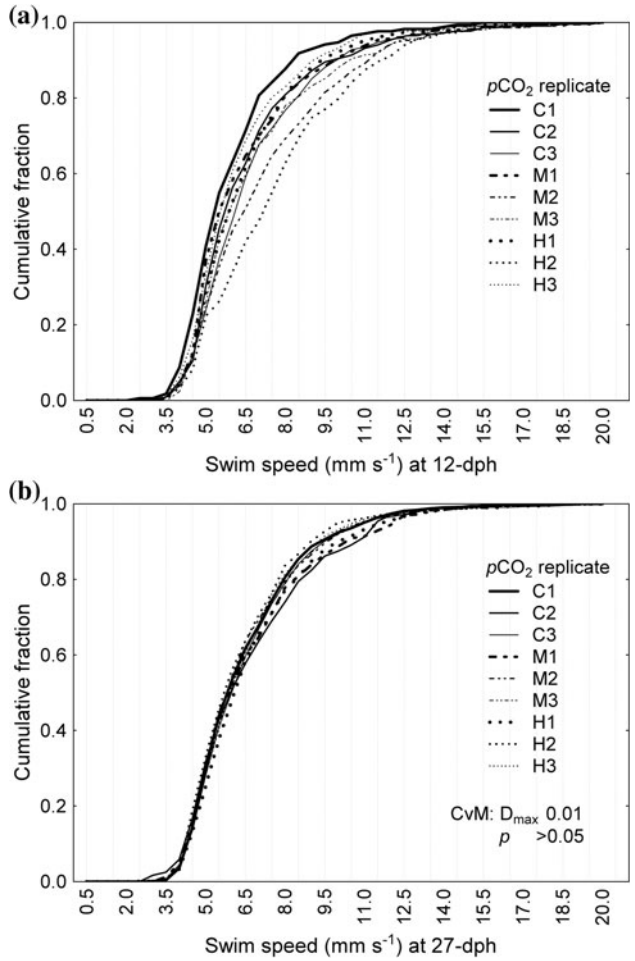

Fig. 5 Cumulative fraction plots for the Kolmogorov–Smirnov tests of swim speed of Atlantic cod larvae (*Gadus morhua* L.) at **a** 12 and **b** 27 dph under three pCO_2 levels (C = 370 µatm, M = 1,800 µatm, and H = 4,200 µatm). Within-treatment variability in all the groups at 12 dph and H-group at 27 dph prevented pairwise treatment comparison

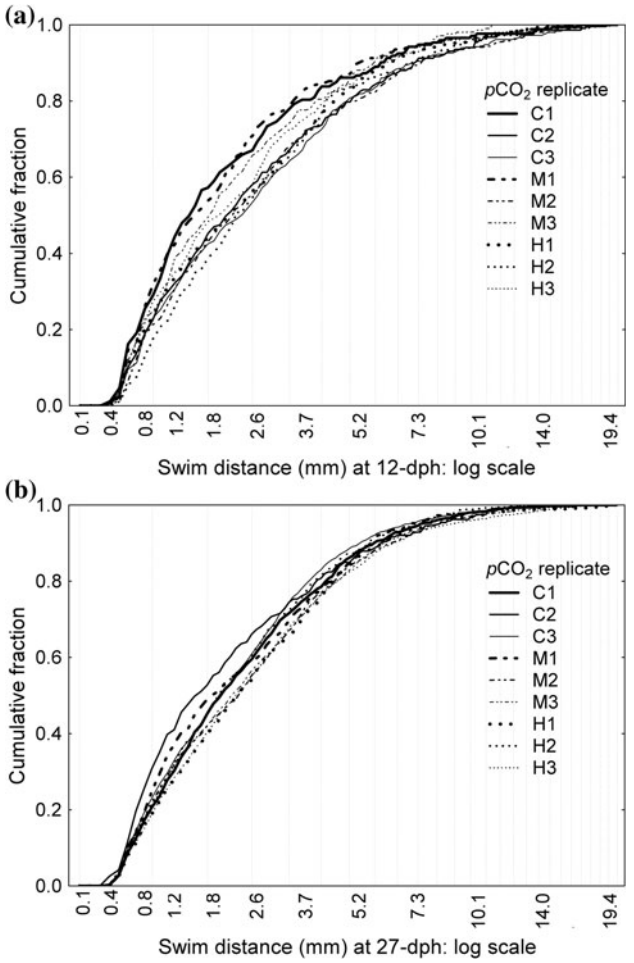

Fig. 6 Cumulative fraction plots for the Kolmogorov–Smirnov tests of swim distance of Atlantic cod larvae (*Gadus morhua* L.) at **a** 12 and **b** 27 dph under three pCO_2 levels (C = 370 µatm, M = 1,800 µatm, and H = 4,200 µatm). Within-treatment variability in the C- and M-groups at 12 dph and C- and H-groups at 27 dph prevented pairwise treatment comparison

10° vertical half-angle, prey below and above this field of view are high enough to cause the larvae to either move up or down. The current study reported restrictions in the vertical turn angle of the high-pCO_2 cod larvae at 12 dph; however, we can only speculate about the biological significance of this observation.

The 12- and 27-dph cod larvae used in this experiment did not show significant differences in dry weight, growth rate, or biochemical indices such as RNA/DNA ratio and protein content between pCO_2 treatments (Frommel et al. 2012). Only subtle changes in the swimming behavior of cod larvae were discernible in this study, even at very high concentrations of pCO_2. A possible reason could be that mortality during the experiment had removed individuals most sensitive to elevated pCO_2. Frommel et al. (2012) reported significant histological damage in the liver, pancreas, kidney, eye, and the gut of cod larvae from the medium and high treatments of this experiment at 32 dph. The tissue damage was not present in the 46-dph cod larvae, suggesting death of tissue-damaged individuals. The impacts of elevated pCO_2 can be reduced when food supply is high, for example, in blue mussels (Melzner et al. 2011) and the coral *Porites* spp. (Edmunds 2011). Therefore, it is also possible that the prey density (2,000 prey L^{-1}) used in our study, which is much higher than those experienced by cod larvae in the field (Tilseth 1984; Ellertsen et al. 1984; Ellertsen et al. 1987), modulated the impacts of elevated pCO_2.

Our results indicate that the swimming behavior of Atlantic cod larvae is resilient to elevated pCO_2. Even at extremely high pCO_2 levels of 4,200 µatm, only minor changes were reported in only two of the 12 swimming kinematic variables observed in 12- and 27-dph cod larvae. The minor changes observed in stop duration and vertical

turn angles could subtly affect the ability of Atlantic cod larvae to modulate their foraging behavior, but they could also represent a statistically significant result that has no ecological significance. Our results support the previous findings by Melzner et al. (2009) that Atlantic cod juveniles reared for 4 and 12 months in 3,000 and 6,000 µatm of pCO_2, respectively, were not compromised in their locomotory performance. It remains to be seen whether the swimming behavior of Atlantic cod larvae is affected when elevated pCO_2 is coupled with other stressors such as increased temperature, hypoxia, and low food availability (Pörtner et al. 2005; Munday et al. 2009a; Kristiansen et al. 2011; Nowicki et al. 2012).

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