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SHORT NOTE

Vertical distribution of echinoid larvae in pH stratified water columns

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Abstract

The abundance and distribution of many benthic marine organisms are shaped by the success of their dispersive larval life-history stage. An increasing number of studies have shown that ocean acidification negatively impacts the larval life-history stage, including those of echinoids which are commercially and ecologically important. However, little is known about the behavioral responses of echinoid larvae to different pH levels in the water column. Changes in vertical movement in response to the naturally occurring pH variations caused by biological activities and/or physical conditions could affect dispersal and recruitment. In this study, we quantified the vertical distribution of larval sand dollars, *Dendraster excentricus* (Echinodermata), in water columns with stratified layers of seawater varying in salinity and pH. When larval sand dollars swimming upwards in ambient seawater ($\mathrm{pH_{NBS}}$ 7.86 ± 0.04) encountered a layer of low pH ($\mathrm{pH_{NBS}}$ 7.54 ± 0.04) seawater, about half of the individuals (53 ± 28%) were aggregated near the transition layer 60 min after the start of the experiment. Preliminary video analysis showed larvae reversed their direction of travel and altered the shape of their helical swimming trajectories, upon encountering the transition layer moving from ambient to low pH water. In contrast, when larval sand dollars swimming upwards in acidified seawater encountered ambient seawater, they continued to swim upward to aggregate near the top of the column. In control water columns with uniform pH, larvae did not change swimming behavior regardless of whether pH was ambient or acidified and whether salinity was uniform or stratified. These results indicate that stratification itself did not strongly affect the vertical distributions of larvae. These observations suggest that echinoid larvae, and perhaps many other types of planktonic larvae, may use behavioral plasticity to reduce exposure to stresses from ocean acidification. The presence and effectiveness of these responses may improve the ability of larvae to cope with stressful, dynamic habitats, and hence may be significant to prediction of potential impacts of global climate change.

Introduction

Many benthic marine organisms have a biphasic life history, which includes a planktonic larval stage that can last for several days to months. This planktonic phase often confers benefits such as location of food resources, decreased likelihood of inbreeding for the next generation, and dispersal, but may also impose costs such as high mortality due to predation and/or starvation, advection away from favorable habitats, and exposure to physiologically stressful conditions (Chia et al. 1984; Pechenik 1999; Allen et al. 2018). Water columns in nature are often non-uniform and maybe stratified along isopycnals with each layer having distinct chemical properties. Most planktonic larvae have some degree of control over their vertical position in the water column, which in turn affects their long-distance horizontal transport by physical processes (Metaxas and Young 1998; Clay and Grünbaum 2011). For example, many larvae can detect multiple physical,

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chemical, and biological cues and modify their swimming behaviors accordingly. Such behavioral changes could in turn alter their vertical positions in the water column (Arellano et al. 2012; Sameoto and Metaxas 2008a). With growing concerns over global climate change and the potential implications for dispersal (Gallego et al. 2017; Chan et al. 2018; Sorte et al. 2018), it is critical to understand how larvae might use swimming to control vertical distributions in response to changes in their environment.

In echinoderms, changes in larval swimming behavior in response to changing environmental conditions (e.g., salinity, temperature, ocean acidification, food patches at different larval stages) are well established (Metaxas and Young 1998; Sameoto and Metaxas 2008a; Chan and Grünbaum 2010; Chan et al. 2011; Daigle and Metaxas 2011). For example, video motion analysis showed that larval *Dendraster excentricus* altered the shape of their swimming trajectories to maintain vertical velocity when exposed to colder water (20 °C vs 12 °C; Chan and Grünbaum 2010). These larvae were also observed to avoid swimming across a large salinity discontinuity (15 psu overlaying 31 psu) and to aggregate above food patches (Arellano et al. 2012). However, responses vary across taxa. In larvae of the sea urchin *Strongylocentrotus droebachiensis* and the sea star *Asterias rubens*, the differences in temperature between water layers (i.e., a sharp thermocline) did not affect the vertical distribution (Daigle and Metaxas 2011).

Ocean acidification (OA), the reduction in surface ocean pH due to the increased uptake of atmospheric carbon dioxide (CO_2) is predicted to become more widespread and intense. Many coastal areas, however, have already experienced natural pH fluctuations with magnitudes comparable to future open ocean predictions. Not only does pH change with diurnal and seasonal patterns due to chemical, biological and physical activities (Guadayol et al. 2014; Murray et al. 2015; Boyd et al. 2016; Lowe et al. 2019), it can also vary across space both vertically and horizontally. Vertical stratification in pH has been observed over few centimeters to several meters, such as those observed in thin layer structures within the water column (Dekshenieks et al. 2001; McManus et al. 2003), in coastal vents (Kerrison et al. 2011), in seagrass meadows (Hendriks et al. 2014; Cyronak et al. 2018) and coral reefs (Guadayol et al. 2014). Spatial variability of pH was recorded in nearshore California kelp forest with mean pH 8.07 at 7 m depth and mean pH 7.87 at 17 m depth (Frieder et al. 2012). Similarly, in Ryder Bay, West Atlantic Peninsula, glacial meltwater and phytoplankton blooms in the summer resulted in a high surface pH water (8.21–8.48) and the settling organic debris and remineralization induced reduction of pH (7.84–7.87) in deep waters (Jones et al. 2017). Vertical gradients in pH were also observed in the US Northeast Shelf with the aid of glider pH sensor (Saba et al. 2018).

In the Salish Sea, WA, USA, where the study takes place, pH is typically high in surface waters, while subsurface waters often have more acidic pH (Feely et al. 2010). In the Hood Canal Basin, for example, a strong vertical pH gradient was observed, pH ranged from 7.77 to 8.25 in the shallow surface waters (< 8 m depth) during summertime and underlying waters had pH values from 7.32 to 7.75 (Feely et al. 2010). Distinct thin layers occurred between 1.4 m and ~ 24 m depth with 0.12–3.6 m thickness in East Sound, WA (Dekshenieks et al. 2001). pH levels within these layers could decrease sharply from pH 8.15 at 5 m depth to 7.74 at ~ 24 m (Hanson and Donaghay 1998). Therefore, vertically swimming and migration plankton could already be exposed to near-future pH conditions at present.

While an increasing body of work demonstrates the negative impacts of OA on a diversity of marine taxa (Kurihara 2008; Espinel-Velasco et al. 2018), little is known about the effects of pH on larval swimming and the movement of larvae within water columns varying in pH (Chan et al. 2018). By altering vertical position in response to OA, larvae could increase or decrease their short-term exposure to pH stress, ultimately affecting their survival, development and long-range dispersal. In this study, we attempt to gain insight into behavioral regulation of pH exposure by experimentally testing the hypothesis that echinoid larvae alter swimming behavior to reduce exposure to OA stress.

According to the climate variability hypothesis, populations that are found in more variable environments are better adapted for coping with conditions predicted under future climate change (Gaitán-Espitia et al. 2017). An implication of this hypothesis is that species intermittently exposed to low pH may be most likely to exhibit beneficial responses or tolerance to OA. The sand dollar *D. excentricus* dwells on sandy bottoms within sheltered bays, lagoons and coastal areas in the East Pacific region (Emlet 1986) and their larvae have been documented at intermediate depth (6 m) or deeper (10–15 m) from surface water (Emlet 1986; Pennington and Emlet 1986). These habitats are influenced by seasonal upwelling and depth-dependent pH fluctuations (Feely et al. 2008). *D. excentricus* is highly abundant (Merrill and Hobson 1970) and is key benthic suspension feeder that consumes a large amount of small prey and other settling larvae (Timko 1976). These broadcast spawners release gametes into the water column that develop into plutei which remain planktonic for 5–6 weeks, depending on temperature. These plutei have ciliated arms for swimming and feeding and were shown to modify vertical swimming behavior in response to other environmental cues (Pennington and Emlet 1986; Arellano et al. 2012). For example, the larvae of *D. excentricus* underwent diel vertical migration (DVM), descending during the daytime below the harmful levels of ultraviolet light. Temperature, thermal discontinuities and visible light, however, did not cause the observed DVM

(Pennington and Emlet 1986). Therefore, we hypothesized that larval swimming behaviors, and hence vertical distributions, of *D. excentricus* would change in stratified water columns depending on the pH of the layers. More specifically, we hypothesized that larvae of *D. excentricus* would move towards layers with lower pH stress, or avoid moving into layers with greater pH stress, resulting in lower larval density in acidified water layers.

Materials and methods

Larval cultures

Adult individuals of the sand dollar, *Dendraster excentricus,* were haphazardly collected and handpicked from the intertidal during low tide at Ship Bay on Orcas Island, Washington, and maintained in tanks with flowing seawater at Friday Harbor Laboratories (FHL). Injection of 0.5–1 ml of 0.55 M KCL into the coelomic cavity induced spawning of gametes. Ova (from three females) were washed twice with filtered seawater and placed in a 1000 ml beaker and then fertilized with motile sperm (~ 1000 sperm ml^{-1}) from two males. Fertilization success was confirmed by the presence of fertilization envelopes 2–5 min after fertilization. Seawater was changed twice in the 1st h after confirmation of > 95% fertilization success, to remove excess sperm. Developing embryos and larvae were reared in 4 l culture jars of filtered seawater (0.45 µm) at 12 °C, with stirring by the apparatus described by Strathmann (1987). A complete water change was performed every other day. Due to the limited time and availability of acidified treated water, only one stage (four-arm pluteus) was assessed in the study. The four-armed stage (3–4 days post-fertilization) is a good compromise, because (1) the availability of previously published data on swimming behaviors at this stage (Chan and Grünbaum 2010), and; (2) four-armed plutei lack muscular control of their arms and arm-flexing behaviors are suggested to help redirect swimming orientation of echinoid larvae (Lacalli and Gilmour 1990; MacNeil et al. 2017). By focusing on an earlier developmental stage, we reduced another source of variability.

Salinity and pH measurements

Acidified seawater (ASW) was obtained from the FHL Ocean Acidification Environmental Laboratory (OAEL). Seawater was filtered (0.2 µm), UV sterilized, stripped of CO_2, and released into large coolers as seawater reservoirs. The ASW was prepared by constant addition of pure CO_2 and CO_2-free air into the coolers controlled by a UDA2182 process controller (Honeywell, Phoenix, Arizona). Ambient filtered seawater (FSW) was obtained from flow-through systems from the surrounding bay. The pre-calibrated ASW and FSW were then collected in airtight jars and transferred to the experimental chambers (refer to the experimental design section below). The pH levels used in the experiment (Table 1) were based on the ambient pH in Friday Harbor, San Juan Island (~ 7.9) and the predicted estimated decrease in pH levels to 7.3–7.5 by the year 2300 (Caldeira and Wickett 2003).

Stratified experimental water columns were constructed such that the lower density layer (salinity of 25‰) stayed above and distinct from the denser bottom layer (ambient salinity, 30‰). Low salinity water was prepared by mixing reverse osmosis freshwater with ambient FSW/ASW. Salinity was measured using a hand-held refractometer. pH_{NBS} of the pre-calibrated water was measured prior to introduction

Table 1 Seawater carbonate chemistry parameters of all treatments

	Measured			Calculated		
Treatments	pH_{NBS}	S (‰)	T (°C)	pCO_2 (µatm)	Ω_{Ar}	Ω_{Ca}
ASW top	7.56 ± 0.03	25	12	1326 ± 97	0.59 ± 0.04	0.95 ± 0.06
FSW bottom (Treatment 1)	7.83 ± 0.01	30	12	652 ± 24	1.18 ± 0.04	1.87 ± 0.06
FSW top	7.91 ± 0.01	25	12	565 ± 11	1.23 ± 0.02	1.99 ± 0.03
ASW bottom (Treatment 2)	7.51 ± 0.02	30	12	1435 ± 77	0.59 ± 0.03	0.94 ± 0.05
FSW (control)	7.90 ± 0.07	30	12	556 ± 92	1.36 ± 0.18	2.16 ± 0.29
ASW (control)	7.50 ± 0.03	30	12	1465 ± 92	0.58 ± 0.03	0.92 ± 0.05
Salinity, high (control)	7.86 ± 0.06	30	12	613 ± 82	1.25 ± 0.15	1.99 ± 0.24
Salinity, low (control)	7.88 ± 0.03	25	12	615 ± 41	1.15 ± 0.06	1.86 ± 0.10

Seawater pH, temperature and total alkalinity (2096.60 ± 0.09 µmol kg^{-1}) of ambient filtered seawater (FSW) and acidified seawater (ASW) were measured and used to calculate CO_2 partial pressure (pCO_2), aragonite (*Ω*ar) and calcite (*Ω*ca) saturation states using the package *seacarb* for *R*

All values are expressed as mean ± SD

Seawater pH values were pooled from the two measurements obtained before the start of the experiment and after the experimental trials to the chambers and sub-samples of the top layer for each chamber were collected and re-measured at the end of each trial. pH between the first and second measurements differed less than 0.03–0.05 unit, indicating a stable pH layer within the 1 h duration of the experiment. pH was measured with a pre-calibrated benchtop pH meter (WWR Scientific 800). Total alkalinity from control ambient water ($n = 3$, measured by open cell titration method), pH_{NBS}, salinity and temperature were used to calculate CO_2 partial pressure (pCO_2), aragonite (Ω_{Ar}) and calcite (Ω_{Ca}) saturation states using the package *seacarb* for *R*. All carbonate chemistry measurements were pooled and are presented in Table 1.

Experimental design

We observed the swimming behaviors and quantified the vertical distribution of four-armed plutei within stratified water columns that varied in salinity and pH. Two stratified pH treatments conditions were applied, with nine replicates each. Treatment 1 had a bottom layer consisting of ambient FSW (pH_{NBS}; mean ± SD = 7.83 ± 0.01, $n = 9$, 30‰), and a top layer of ASW (pH_{NBS}; mean ± SD = 7.56 ± 0.03, $n = 9$, 25‰). A reciprocal treatment (Treatment 2) had a bottom layer of ASW (pH_{NBS}; mean ± SD = 7.51 ± 0.02, $n = 9$, 30‰) and a top layer of FSW (pH_{NBS}; mean ± SD = 7.91 ± 0.01, $n = 9$, 25‰). As controls, we additionally conducted six replicates each of uniform columns of ASW (pH_{NBS}; mean ± SD = 7.50 ± 0.03, $n = 6$, 30‰) and of ambient FSW (pH_{NBS}; mean ± SD = 7.90 ± 0.07, $n = 6$, 30‰), and six replicates of stratified columns of FSW with two salinity levels (bottom layer, pH_{NBS}; mean ± SD = 7.86 ± 0.06, $n = 6$, 30‰ and top layer, pH_{NBS}; mean ± SD = 7.88 ± 0.03, $n = 6$, 25‰).

A water-jacketed acrylic tank with four chambers (3.5 × 3.5 × 30 cm each) was used for observing larval swimming and distributions (Fig. 1). The water jacket was filled with freshwater to maintain constant 12 °C temperature in the chambers during the experiment. For stratified treatments, the top layer (i.e., ambient FSW or ASW with 25‰) was added first, followed by the bottom layer (i.e., ambient FSW or ASW with 30‰), through plastic tubing (0.4 cm diameter) attached to the bottom of each chamber (Fig. 1). Water was introduced slowly into the chambers to prevent mixing between the top and bottom layers. To minimize larval shock, four-armed plutei (200–300 individuals) were acclimated for over 5 min in 50 ml with their respective bottom layer treatment before being introduced to the same bottom layer treatment in the column. After acclimation, the larvae were introduced gently through the same plastic tubing, which is 20 times larger than larval body length to avoid excessive shear stress (following Chan et al. 2011). The number and positions of the larvae were visually determined by the same observer for consistency, while another researcher served as data recorder and documented the observer's counts. Tick marks at 1-cm increments were pre-drawn on the 30-cm tall chamber. The ~ 200 µm larvae were visible to the naked eye when illuminated with a white LED

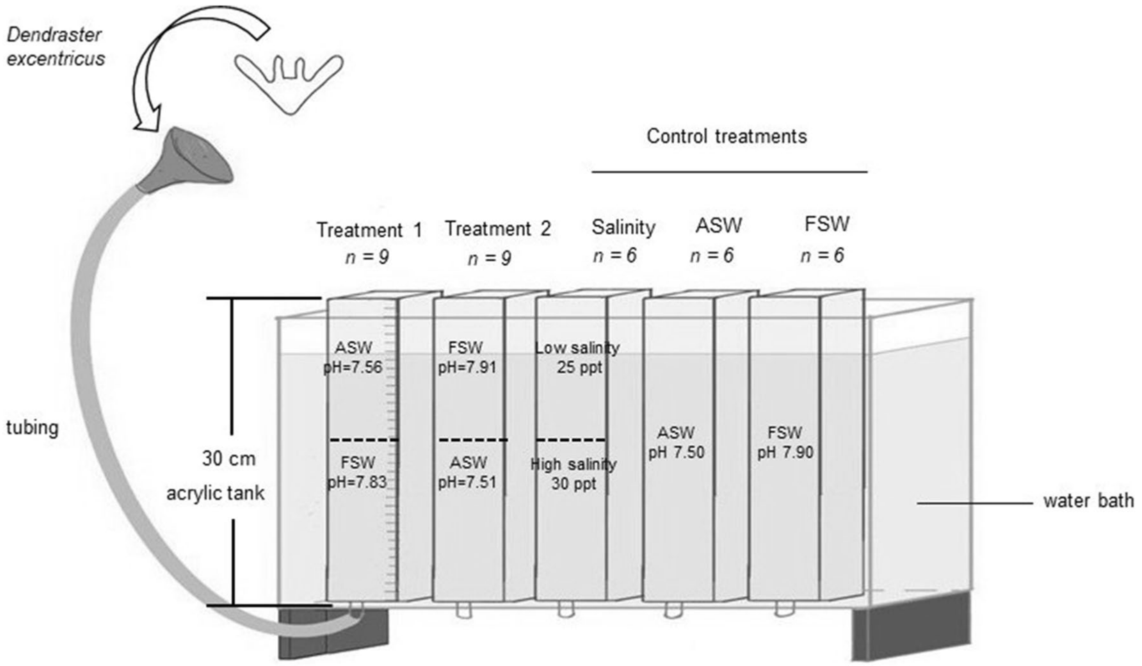

Fig. 1 A schematic representation of the experimental design. Number of replicates *n* were performed for several times within the same day for each experimental treatment. Larvae were pre-incubated in the bottom water conditions before being added to the column

flashlight from the side against a dark background. Larval abundance was recorded with respect to depth at around 5, 30, and 60 min after larvae were introduced into the chamber. To ensure the exposure duration was similar between treatments, larvae were introduced to the chambers in a staggered manner (2 min apart). Each experimental column was considered to be a replicate, and the experiments per treatment were repeated several times within the same day to achieve the desired number of replicates. Due to logistical constraints and the tendency for sand dollar larvae to swim upwards in water columns (Podolsky and Emlet 1993; Pennington and Emlet 1986), no trials were conducted in which larvae were introduced to top of the column.

Statistical analyses

The center of larval mass (ZCM), a metric quantifying the height or depth (cm) distribution of larvae within a column at a given time, was calculated with the formula (Daigle and Metaxas 2011):

$$\mathrm{ZCM} = \sum p_i z_i.$$

where p_i represents the proportion of larvae at within depth interval i, and z_i is the median interval depth, for all depth intervals i. ZCM was analyzed with repeated-measures ANOVA (Statistica 7.0) with treatments as main effect and time (minutes) as the repeated measure. To determine whether *ZCM* had stabilized, a Tukey's HSD test was performed for time.

Results

In Treatment 1, 5 min after gently introducing larvae into the bottom of the 30 cm tall column, approximately 55 ± 17% (% mean abundance ± SD) of larvae had accumulated at the transition layer between ambient filtered sea water (FSW, $\mathrm{pH_{NBS}}$ 7.83 ± 0.01; mean pH ± SD) and acidified sea water (ASW, $\mathrm{pH_{NBS}}$; 7.56 ± 0.03, Fig. 2a). After 30 min, larvae had maintained their vertical distribution at the interface between the stratified layers at around 15–20 cm (Fig. 2a). After 60 min, 17 ± 15% of the larvae were observed near the top of the column, while 53 ± 28% of the larvae were found near the interface between the stratified layers. In Treatment 2, larvae were evenly distributed throughout the column after 5 min. After 30 and 60 min, the distribution of the larvae shifted towards the top in the FSW layer ($\mathrm{pH_{NBS}}$ 7.91 ± 0.01, Fig. 2b).

In the ambient FSW unstratified controls ($\mathrm{pH_{NBS}}$ 7.90 ± 0.01; mean pH ± SD), 90–93% of the larvae were present in the upper part of the water column and swam towards the surface 5 min after introduction (Fig. 2c). In the ASW unstratified controls ($\mathrm{pH_{NBS}}$ 7.50 ± 0.03), larvae swam towards the surface after being introduced into the columns (Fig. 2d). The stratified salinity controls ($\mathrm{pH_{NBS}}$: 7.86 ± 0.06 and 7.88 ± 0.03; salinity: 25 and 30‰, respectively) showed even distributions throughout the columns 5 min post-introduction, followed by movement of larvae to the top of the column at 30 and 60 min (Fig. 2e).

The center of larval mass (ZCM) showed significant differences among treatments (ANOVA, $F_{4,\,31} = 9.116$, $P = 0.0001$) and time (ANOVA, $F_{2,\,62} = 69.594$, $P < 0.0001$) as well as treatment–time interaction (ANOVA, $F_{8,\,62} = 2.775$, $P = 0.0109$, Fig. 3). In Treatment 1 (ASW on top), the ZCM (17.4 ± 1.3 cm; mean height ± SD) was stable with no significant differences in ZCM over time (Tukey test, $P > 0.05$). The ZCM of the controls (FSW, ASW and salinity) and Treatment 2 (FSW on top) at 60 min were located significantly closer to the surface of the column than that of Treatment 1 (Fig. 2b–e).

Video tracking of individual larvae in a representative column suggests that the larvae in Treatment 1 swam upwards crossing the transition layer between the two water layers before aggregating at the transition layer. Larvae in this treatment exhibited distinctive swimming patterns, e.g., direction reversal and altering the pitch of helical (corkscrew-like) paths, when approaching the pH/salinity transition layer (Fig. S1).

Discussion

Thermal and salinity gradients in the water column can affect the vertical distributions of marine benthic larvae, which in turn influence dispersal (Sameoto and Metaxas 2008a; Daigle and Metaxas 2011). While there is increasing concern about the impacts of ocean acidification on key ecological taxa such as echinoids, little is known about the role of pH in shaping vertical distribution of their planktonic larvae. We hypothesized that echinoid larvae respond to changes in pH, such as those naturally occurring in their present-day habitats, by modulating their vertical swimming behaviors. We further hypothesized that swimming responses enable these larvae to avoid or reduce exposure to stressfully low pH layers in stratified water columns. Here, we used larval sand dollar *Dendraster excentricus*, which normally have a bias towards upwards swimming (Pennington and Emlet 1986), as a model system in which to test these hypotheses. Our observations show that normally up-swimming larvae aggregated in the vicinity of the ~ 5 cm transition layer at which acidified water overlaid ambient pH seawater. The observed aggregations suggest these larvae stopped or greatly reduced upward swimming. These observations are consistent with our behavioral hypotheses and suggest shifts in in situ water column positions that could

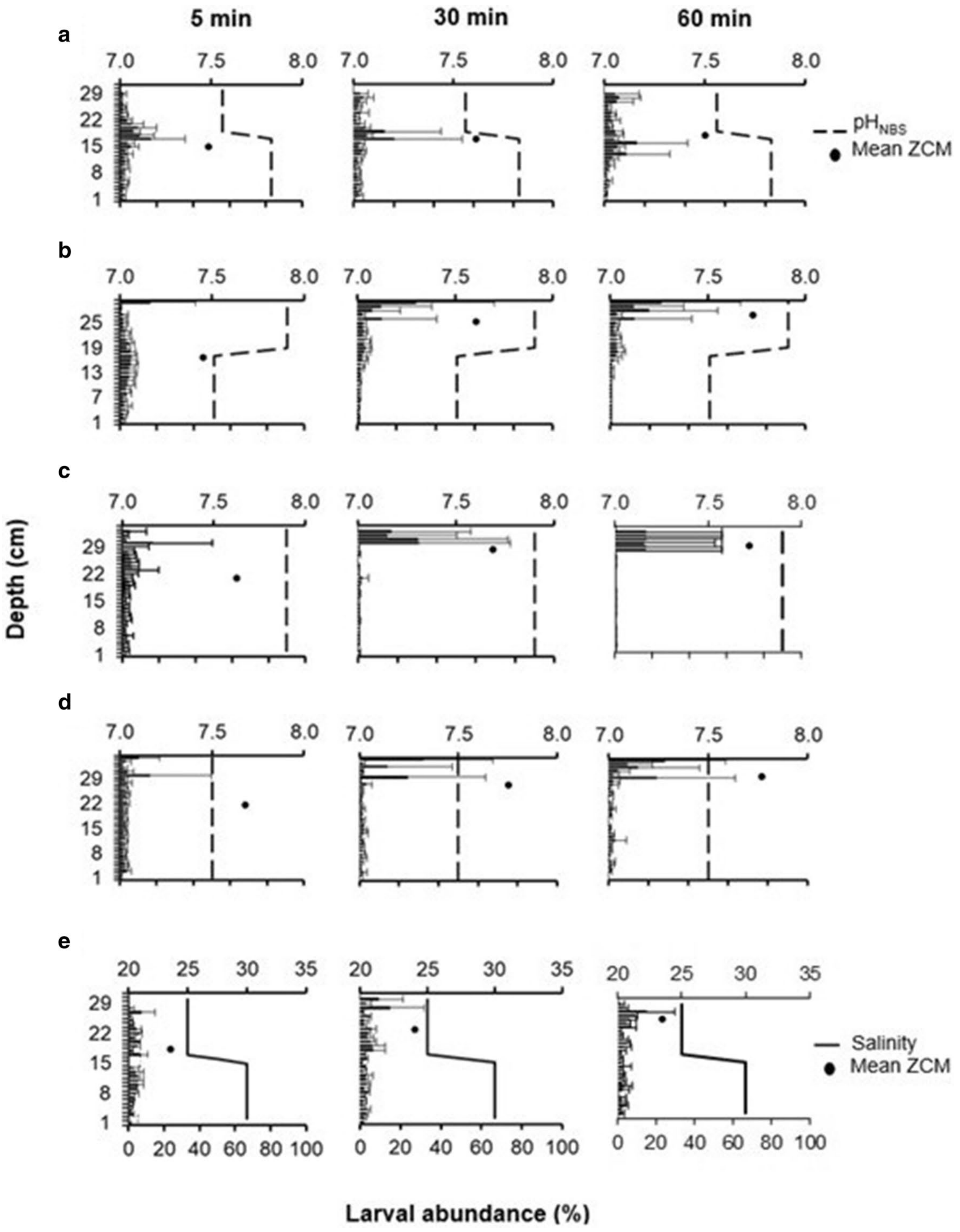

Fig. 2 Vertical distribution of four-arm pluteus larvae of *Dendraster excentricus* after 5, 30 and 60 min in stratified water columns of **a** ambient FSW bottom, ASW top (Treatment 1), **b** ASW bottom, FSW top (Treatment 2); **c** unstratified FSW control, **d** unstratified ASW control and **e** stratified salinity control. All stratified columns **a**, **b**, **e** had 25‰ overlaying 30‰. Bars represent mean larval abundance, with error bars for (plus/minus) standard deviations; **a**–**b**: $n=9$, **c**–**e**: $n=6$; ZCM (black dots) indicates the center of larval mass

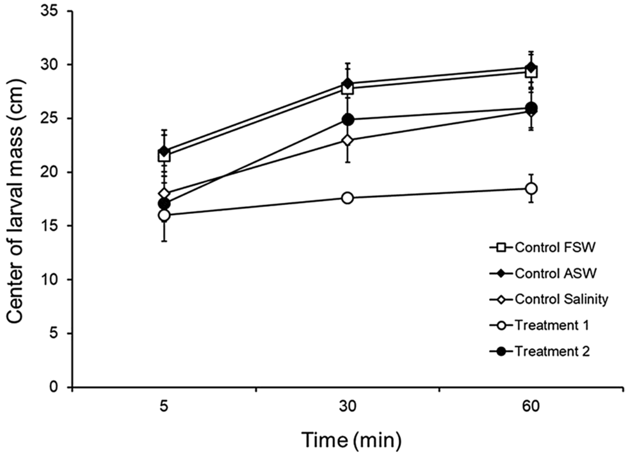

Fig. 3 Center of larval mass (ZCM; see text for details) over time of four-arm plutei larvae of *Dendraster excentricus*. Error bars represent standard error of means (Treatment 1 and 2; $n=9$; FSW, OA and salinity controls; $n=6$)

reduce larval exposure to OA stresses, but could also alter normal patterns of life history.

The larvae of *D. excentricus* are known to behaviorally respond to diverse environmental cues (Pennington and Emlet 1986; Podolsky and Emlet 1993; Arellano et al. 2012). Our results further suggested that the larvae of *D. excentricus* prefer the water stratum with ambient pH over those that with a lower pH when presented with a choice. This behavior was observed in the controls (uniform water columns of FSW and ASW), in which larvae aggregated within the surface layer (1–5 cm from the top) at the end of an hour. When acidified water (ASW) was layered on top of ambient FSW in a stratified column (Treatment 1), larvae accumulated at the transition layer, favoring FSW and avoiding ASW. However, when ASW was on the bottom (Treatment 2), larvae aggregated in the top FSW layer, again avoiding ASW. Similarly, in water columns stratified by salinity alone, larvae swam across the transition layer and continued upwards (Fig. 2e). Interestingly, our results differed from earlier reports in which larval sand dollar aggregation at the halocline boundary took place when the salinity difference was exceeding 10‰ (31‰ and 19/17‰; Arellano et al. 2012). Similar patterns of aggregation have been reported for other marine benthic fauna (e.g., sea star *Asterias rubens* and sea urchin *Strongylocentrotus droebachiensis*) at these > 10‰ sharp halocline (Sameoto and Metaxas 2008b). The salinity difference used in our study was only 5‰, within a range frequently experienced by *D. excentricus* in situ. This may be the reason larvae in our study behaved differently than in these previous studies (Sameoto and Metaxas 2008b; Arellano et al. 2012). Results from control columns suggest that the larval aggregation observed in Treatment 1 (bottom layer of ambient FSW and a top layer of ASW) was not likely due to the salinity differences, and was more likely due to the pH differences.

Preliminary observations using video motion analysis were consistent with the hypothesis that larvae are capable of detecting and responding to the pH of their surrounding waters by changing their swimming (Fig. S1), and hence altering their vertical distributions (Fig. 2a). Interestingly, this contrasts with the response of larval *D. excentricus* to temperature decrease, in which the relative vertical component of swimming was increased to maintain up-swimming despite decreased swimming speed (Chan and Grünbaum 2010). Therefore, the observed larval behavior in the present study does not appear to be a generic stress response. Tight zigzags that represent constant swimming were observed over most of the 16-min video, suggesting an extensive oscillatory horizontal movement with very little vertical movement. This result indicates larvae were still swimming but reducing the vertical component of their swimming velocity, preventing entry into the low pH layer. The observed larval aggregation at the transition layer might also indicate increased frequency of larval hovering or changes in swimming direction. Although these behavioral responses were not quantified in this study, they are worth exploring in future experiments.

Ectoderm of larval echinoids is thin and leaky, offering high permeability for ions and potentially a rapid equilibration of extracellular pH to the surrounding seawater pH (Stumpp et al. 2012). Such equilibration suggests a variety of physiological mechanisms through which larval sand dollars might sense pH variations over short periods of time. Larval echinoids are calcifying organisms with relatively alkaline gut conditions, for which maintaining pH homeostasis in the larval gut and forming the primary skeletal mesenchyme cells (PMCs) could be energetically costly (Pan et al. 2015; Hu et al. 2017, 2018). These physiological characteristics suggest mechanisms through which changes in vertical distributions might be a beneficial active avoidance to reduce pH stress under present and future oceanic conditions.

The mechanisms underlying swimming behavior changes in response to pH are still unknown. Larval sand dollars reared in elevated $p\text{CO}_2$ levels developed narrower bodies with smaller stomachs and longer arms (Chan et al. 2011). The larvae used in this experiment, however, were reared under normal pH and were extracted from the same batch cultures immediately prior to the experiments. Therefore, the observed changes in larval distribution and swimming were not due to morphological differences between pH treatments. It is possible that ciliary motion (e.g., beat plane direction, angular velocity and beat period) was affected by the pH variations experienced over short periods of time (< 1 h). Ciliary motion in larval urchins is regulated by various neurotransmitters, including serotonin, dopamine, and γ-aminobutyric acid (GABA)

(Wada et al. 1997; Katow et al. 2016). Interestingly, reduction in pH has been shown to affect neurotransmitter functions, especially GABA receptors, in other marine organisms (Clements et al. 2017; Watson et al. 2017). Reduction in pH might have compromised neurotransmitter function affecting the ciliary motion in the larval sand dollars in this study. However, the roles of these GABA receptors in altering larval behaviors and vertical distributions both generally and in response to pH warrants further studies.

While our findings are consistent with the hypothesis that larval echinoids actively avoid low pH waters, further experimental investigations are needed. It remains unknown whether the planktonic larvae of other benthic animals display avoidance towards low pH water. One key question raised by our experiments is how the observed behaviors translate into larval vertical distributions in the field. A combination of additional behavioral observations, numerical modeling and field sampling could be used to assess the hypothesis that larvae avoid low pH water strata in their natural environments. Another question is whether the observed response is maintained through ontogeny, spawning season, between parental lineages and populations (see Hodin et al. 2018 for a discussion on within-species variations in behavioral responses to turbulence). Changes in pH responses across each of these factors could translate into distinct implications for differential dispersal. Our observations also highlight new research questions into the mechanisms of sensory–motor or ciliary control associated with pH changes, e.g., the involvement of GABA receptors.

While this study was conducted in a laboratory setting, analogous conditions can be found in the natural environment. Upwelling zones along the western North American coast bring cold, dense, seawater rich in CO_2 up to the surface near the coastlines during the summer months (Feely et al. 2008). If the observed behaviors apply to field conditions, the larval vertical distribution of marine benthic animals and, hence, recruitment patterns could change over short periods of time (weeks) depending on intensity of coastal upwelling.

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Compliance with ethical standards

Conflict of interest All authors declare no conflict of interest.

Ethical approval All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

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