Oxygen conditions affect the swimming behavior of sand dollar larvae

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ABSTRACT

Larval swimming behavior is not only critical to the survival and dispersal of marine invertebrates, but also has larger implications for population dynamics in species with planktonic larvae. However, little is known about these behaviors under variable oxygen conditions. Prior works present conflicting results, with marine invertebrate larvae demonstrating either increased or decreased swimming speeds under low-oxygen conditions when compared to normoxic conditions. Low-oxygen conditions, or hypoxia, can range from 2-5 mg of oxygen per liter of water depending on the group defining the standard (Gobler & Baumann, 2016). Normoxic conditions are anything over 7-8 mg of oxygen per liter of water. Hypoxic conditions are common among coastal zones and will expand with climate change (Gobler & Baumann, 2016). We used video techniques to track the vertical swimming speed of sand dollar *Dendraster excentricus* larvae in hypoxic, normoxic, and stratified conditions. Results demonstrated an increase in vertical swimming speed in hypoxic conditions, while swimming in stratified conditions showed no effect. This implies that larvae are capable of responding to hypoxic conditions by way of escaping low-oxygen environments. These results demonstrate that low-oxygen conditions could cause an escape behavior response and therefore should be considered when modeling larval survival and dispersal.

INTRODUCTION

Larval forms of marine invertebrates are a fascinating study subject with great significance due to their unique life histories, ecological roles and swimming behavior. Larval swimming can lead to greater dispersal which is hypothesized to confer many benefits, such as settlement in more advantageous habitat (Strathmann, 1980) and enhanced habitat selection as larvae have the

opportunity to test a wider diversity of substrata (Obrebski, 1979). On the other hand, larvae may be transported to unsuitable habitats by swimming into parts of the water column with different currents and cues that may affect their dispersal patterns (Strathmann & Branscomb, 1979). Swimming can also serve as a form of predator escape for larvae (McPeek et al., 1996) as well as a method of regulating their vertical position in the water column, allowing them to find food in the case of feeding larvae (Chia et al., 1984).

Despite the significance of swimming in the larval stages of many marine organisms, a deeper understanding of how swimming is affected by environmental conditions is vital to comprehend how future oceanic and climate phenomena may affect larval swimming behavior. Low oxygen zones are commonly found in marine ecosystems (Gobler & Baumann, 2016), and salinity may vary in the ocean due to processes such as precipitation, evaporation, and runoff (Talley, 2002). Oxygen and salinity levels in the ocean could have noticeable effects on larval behaviors like swimming, yet such studies are limited in marine invertebrate larvae, and even fewer have investigated the compound effect of salinity and oxygen stress on larval swimming behavior. The brown bryozoan (*Bugula neritina*) spent less time looking for habitats and delayed settlement when placed in water with low oxygen levels (Lagos et al., 2015). Lagos et al. (2015) also found that *B. neritina* actively avoided hypoxic water. Fertilization success and embryogenesis for the tube forming serpulid worm, *Hydroides elegans*, was reduced in hypoxic water (Leung et al., 2013). Such work would greatly enhance our understanding of species responses to abiotic factors and how animals adapt to multi-factorial effects on physiological performance (Lange & Marshall, 2017).

We used a video-tracking technique to quantify the larval swimming behavior of the sand dollar *Dendraster excentricus* in response to changes in oxygen and salinity levels. A similar

video-tracking technique was used by Chan and Grünbaum (2010) to study larval swimming in *D. excentricus* in response to variation in temperature and diet. Video-tracking is favored for behavioral studies because it removes the necessity for tethering and allows for the use of observation chambers with minimal wall effects (Chan & Grünbaum, 2010). *D. excentricus* larvae serve as a good model organism for the study of swimming behavior due to their ciliary swimming (Strathmann, 1975) and representation of two common larval morphologies, spheroidal in early embryonic development and "armed morphology" in later pluteus stages (Chan, 2012). Moreover, sand dollars are easily cultured and have a well-studied growth and developmental ecology (Chan, 2012).

D. excentricus is found in dense aggregations on sandy bottoms and tidal channels on the western coast from Baja California, Mexico to Southern Alaska (Merrill & Hobson, 1970). Sand dollars play an important ecological role as several organisms rely on sand dollar beds as substrate and shelter from predators (Merrill & Hobson, 1970). Sand dollars are also prey for organisms such as crabs, sea stars, and fishes, and therefore play an important ecological role (Merrill & Hobson, 1970).

We hypothesized that there would be a difference in the swimming behavior between the *D. excentricus* larvae in the normoxic conditions compared to the hypoxic conditions. We predict higher swimming speeds in hypoxic conditions due to a behavioral escape attempt from low-oxygen conditions. Finally, we predict that larvae in environments with both normoxic and hypoxic conditions will actively avoid the hypoxic conditions.

MATERIALS & METHODS

Spawning, fertilization, and larval culturing

Adult *D. excentricus* were gathered from East Sound, Orcas Island, Washington, USA, in spring 2023 and maintained in a flow-through system at Friday Harbor Laboratories, Washington (FHL). 0.5 ml of 0.55 M KCl was injected into the coelomic cavity of one male and one female to induce spawning (Strathmann, 1987). Eggs were collected and placed in a beaker and filled with 0.45-µm filtered seawater, and fertilized with concentrated sperm. Larvae were reared in a 3.7 L jar in 0.45-µm filtered seawater and held in a sea table where temperature was maintained at ~12°C. Water was exchanged with 0.45-µm filtered seawater and fed 5000 cells ml^{-1} of *Rhodomonas* sp. every four days.

Seawater chemistry

Seawater was taken from the seawater system at FHL and filtered to 0.45-µm. DO in seawater was then measured using a DO probe (YSI™ ProODO). As DO averaged 7.30 mg L^{-1} on most experimental days, this concentration was used for normoxic treatments. Because DO fluctuates throughout the day, adjustments were made by either gentle stirring to add oxygen or bubbling in N_2 for oxygen removal. To create hypoxic seawater equal to 2.00 mg L^{-1}, N_2 gas was used to bubble out DO and monitored using a DO probe. This was carried out in 1000-ml tripour beakers, each with 350 ml of filtered seawater. Hypoxic water was then transferred to 500-ml Nalgene flasks by siphon and sealed to prevent re-oxygenation. This process was repeated for seawater at 25 and 30 ppt. To achieve these salinities, salinity was adjusted by adding reverse osmosis (RO) water until the desired concentrations of 25 and 30 ppt were met. A pH probe (Fisherbrand™ pH Pen) was used to sample the water before and after this procedure to assess

changes in pH, which we consider to be a proxy of dissolved CO_2, to ensure that alkalization would not confound results. pH read 8.1 for all samples, with no differences observed in pH before and after bubbling seawater with N_2.

Behavioral observations and video-acquisition

Swimming behavior of six-arm larvae was captured at 12°C in four enclosed Plexiglass chambers measuring 3 × 3 × 11 cm. Each chamber was filled with 250 ml of filtered seawater, corresponding to one of four treatments: 1) hypoxia at 25 ppt, 2) hypoxia at 30 ppt, 3) normoxia at 25 ppt, and 4) normoxia at 30 ppt. We defined a hypoxic treatment as having O_2 concentration of 2.00 mg L^{-1}, and a normoxic control as having a concentration of 7.30 mg L^{-1}. This procedure was replicated the following day. The chambers were then filled with 250 ml of stratified filtered seawater comprising two treatments: 1) hypoxic overlaying normoxic conditions at 25 ppt and 30 ppt, respectively, and 2) normoxic overlaying hypoxic conditions, at 25 ppt and 30 ppt, respectively. Seawater was slowly pumped from below to both ensure minimal mixing and re-oxygenation and create stratified treatments. The water condition that was wanted in the top layer was pumped in first at 25 ppt and after that, the condition wanted in the bottom layer was pumped. Chambers were held in the same water bath and temperature was maintained by a refrigerated and heated bath circulator (Fisherbrand™ Isotemp 4100 R20). 250 larvae were randomly assigned and added to each chamber from below. A camcorder (Sony™ Handycam HDR-CX550) was used to capture swimming near the middle of the tank (10–20 cm), with each video clip capturing the first 10 min of larval behavior.

Analysis

The vertical swimming speeds of the larvae were calculated by replaying video clips and timing the ascent of individuals as they passed through the middle of the tank. The speed of 10 individuals from each of the treatments was recorded. A two-way ANOVA was used to measure the effects of oxygen and salinity on vertical swimming speed, using replicates as blocking factors to account for variation across replicates. Another two-way ANOVA was used to measure vertical swimming speed in the stratified columns, comparing the overlaying oxygen and salinity conditions and the speed of the individuals as they traversed from the bottom position of the frame to the boundary layer and then toward the top position of the frame.

R version 4.2.1 was used to perform these analyses. Plots were created using the *ggplot2* package (Wickham, 2016). The function *shapiro.test()*, a Shapiro test for multiple factors, was used to check for normality of the data and and a Breusch-Pagan test used to check for heteroskedasticity using the *bp()* function in the R package *lmtest* (Achim & Hothorn, 2002). ANOVAs were performed using the R function *aov()*. A post-hoc test was performed using the *emmeans()* function in the R package *emmeans* (Lenth 2023) to determine significant contrasts in treatment levels.

RESULTS

Averages were calculated for the larval swimming speed in conditions that varied by oxygen and salinity levels. Each oxygen and salinity treatment combination had two replicates. In the non-stratified trials, the average larval swimming speeds in normoxic conditions with a salinity of 25 ppt were 0.76 mm s^{-1} and 1.90 mm s^{-1} for the two replicates. In hypoxic conditions (25 ppt), the average speeds were 0.78 mm s^{-1} and 2.64 mm s^{-1}. For normoxic conditions (30 ppt), average swimming speeds were 0.66 mm s^{-1} and 2.40 mm s^{-1}. Hypoxic conditions (30 ppt) featured average speeds of 1.14 mm s^{-1} and 2.22 mm s^{-1}. For the stratified trials, different oxygen conditions were layered by differences in salinity with bottom layers having a salinity of 30 ppt and top layers having a salinity of 25 ppt. When normoxic conditions made up the bottom layer, average swimming speeds were 1.52 mm s^{-1} and 1.10 mm s^{-1} for the two replicates. Normoxic conditions (top layer) featured average speeds of 1.80 mm s^{-1} and 1.44 mm s^{-1}. Average speeds for hypoxic (bottom layer) included 1.66 mm s^{-1} and 1.52 mm s^{-1}. Average speeds for hypoxic conditions (top layer) were 1.42 mm s^{-1} and 1.24 mm s^{-1}.

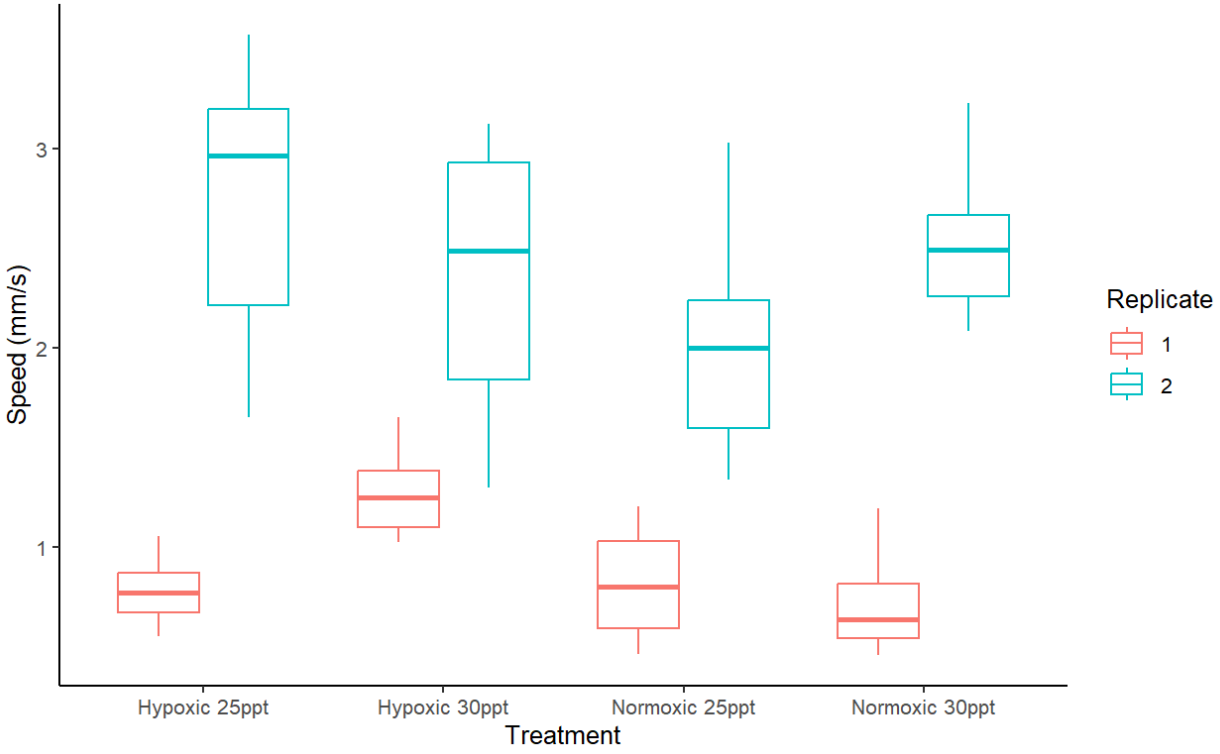
Effects of oxygen and salinity conditions on vertical swimming speed

There were significant effects of oxygen on vertical swimming speed ($p = 0.008$; Table 1) and replicate number ($p < 0.001$; Table 1). The effects of salinity and the interaction of oxygen and salinity were not significant.

Table 1. Two-way ANOVA table for the effects of replicate, oxygen, salinity, and interaction of oxygen and salinity on vertical swimming speed. **Bold** indicates a significant difference ($p < 0.05$).

Source	Sum Sq	Df	F value	Pr(>F)
Replicate	3.789	1	198.781	**<0.001**
Oxygen	0.144	1	7.537	**0.008**
Salinity	0.016	1	0.837	0.363
Oxygen:Salinity	0.008	1	0.430	0.514
Residuals	1.430	75		

Fig. 1. Boxplots of separate replicates. Plots depict the vertical swimming speed of *D. excentricus* larvae under hypoxic and normoxic conditions at 25 and 30 ppt in unstratified columns.



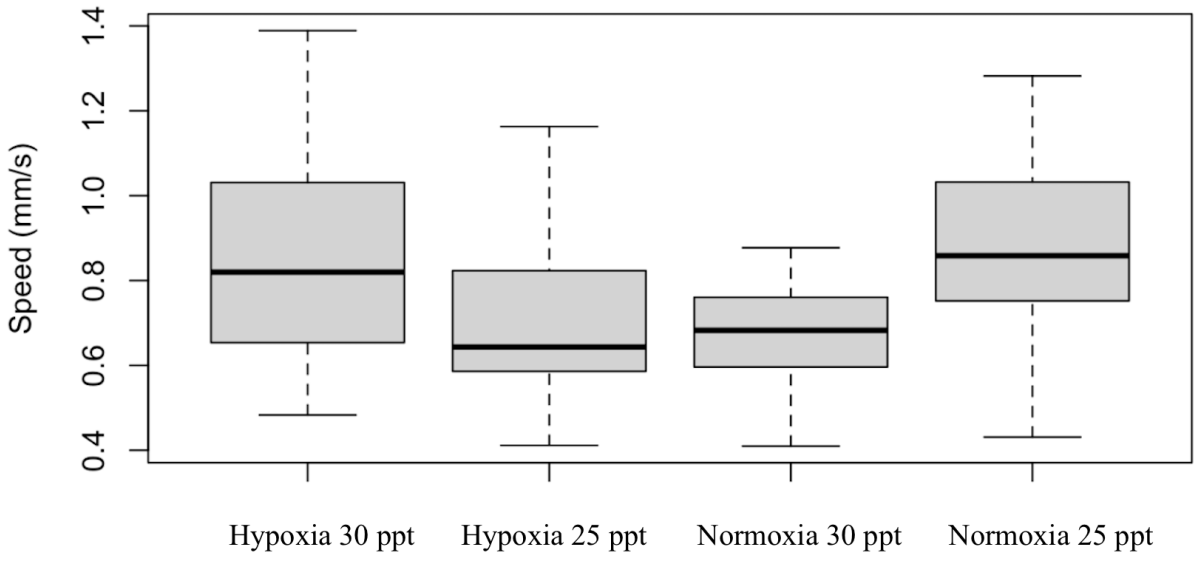
Effects of overlayed oxygen and salinity conditions at different positions

There was no significant effect of overlayed oxygen and salinity conditions on vertical swimming speed (Table 2). No significant effect was observed in swimming speed as larvae crossed the boundary layer from one oxygen and salinity concentration to the next.

Table 2. Two-way ANOVA table for the effects of overlaying oxygen treatments and salinity on vertical swimming speed.

Source	Sum Sq	Df	F value	Pr(>F)
Oxygen	0.003	1	0.052	0.82
Salinity	0.002	1	0.042	0.839
Residuals	3.987	77		

Fig 2. Boxplots depicting the vertical swimming speed of *D. excentricus* larvae under stratified conditions.



DISCUSSION

Marine environments experience fluctuations in abiotic conditions, including oxygen and salinity. The Puget Sound and its adjacent waters in the Pacific Northwest, are an example of a marine system that experiences such fluctuations of these conditions (Deppe et al., 2018). Laboratory experiments were therefore conducted to quantify possible short term swimming responses on the larvae of a local model organism, *D. excentricus*, to changes in oxygen conditions and salinity that are consistent with those that they may encounter in the Puget Sound and adjacent waters (Deppe et al., 2018). Swimming can have important implications for the survival and dispersal of planktonic larvae, two mechanisms that can affect population dynamics. When subjected to different salinities, results from both experiments indicated that larval swimming speed was not affected. This suggests that salinity may not be a decisive factor in larval swimming behavior. When subjected to hypoxic conditions, larvae increased swimming speeds compared to normoxic conditions. This is suggestive of a response to low oxygen conditions, whereby larvae swim to escape such environments. However, larvae demonstrated no significant change in behavior when exposed to stratified layering of different oxygen conditions. Although it was expected that larvae would exhibit different swimming responses in stratified conditions, vertical swimming speed did not significantly change as individuals passed between different oxygen conditions. This may indicate that larvae experience a delayed response to sudden changes in oxygen conditions. Longer observation times, or additional experiments that test for these delayed responses, would be necessary to understand if, and for how long after, exposure to hypoxic conditions affects larval swimming behavior.

There were also significant differences in replicates, with larvae swimming more quickly in the second replicate than in the first. This may have occurred because larvae were older in the

second replicate, and therefore swim faster than younger larvae. These results would be consistent with Chan and Grünbaum (2010), where they observed differences in larval speed in four and eight arm plutei. However, these differences were much less than the differences observed here, and the difference in developmental stage also much greater than the differences in age in this study. Therefore, it is unlikely that larval stage and discrepancies in age impacted the swimming speed of larvae across replicates. An alternative and more likely explanation is that there was increased flow in the second replicate, a consequence of injecting the larvae from the bottom of the column at a faster rate than in the first replicate. This would have increased the speed of the larvae as they traveled up the chamber. Although the rate of injection was different across replicates, this rate was consistent across treatments for each replicate, and thus did not impact the real differences observed in oxygen and salinity treatments.

The results of these experiments contrast studies in other invertebrate taxa, like in nassariid gastropods (Liu et al., 2011) and copepods (Wyeth et al., 2022), where swimming speeds were reduced in low oxygen concentrations. This could be because low dissolved oxygen environments reduce metabolic and physiological activity due to lower rates of respiration. Consequently, larvae would swim at slower rates and remain in oxygen depleted conditions for longer. These conflicting results between our study and the literature suggests that larvae from different taxa may exhibit distinct responses to different oxygen concentrations.

Additional research is required on the topic of larval swimming behavior, especially in the face of fluctuating oxygen conditions. Knowing whether there are changes in the ciliary action of *D. excentricus* larvae while under these conditions would provide valuable information as to how oxygen affects their physiological swimming response, and whether this plays an important role in their observed behavior. Alternatively, low-oxygen conditions may hinder physiological

functions that are not tied to swimming, either triggering or dedicating an increased response in vertical swimming. This study did not measure the prolonged effects of low-oxygen environments, which is necessary to help further our understanding of the behavior and impact of swimming rates of larvae in the field. With climate change, the need for this research is becoming increasingly important as low-oxygen zones are becoming larger and more prevalent (Gobler & Baumann, 2016).

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