Effects of hypoxic layers on the swimming behavior of *Lacuna vincta* larvae from Friday Harbor, WA, USA

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ABSTRACT

Hypoxia has the potential to influence marine organism function at many stages in the life cycle. Most studies on benthic invertebrates have focused on adult animals, but it is important to consider impacts on larvae, as they will have consequences for survival and recruitment of benthic populations. We sought to determine whether hypoxia alters the swimming behavior of *Lacuna vincta* larvae, and whether larvae are able to recognize and avoid hypoxic layers. We used video recording to compare larval behavior in stratified water columns with an upper layer with normoxic oxygen levels (~85% saturation, ~7.5 mg/L) and a lower layer with hypoxic oxygen levels (~13% saturation, ~1 mg/L), to behavior in similarly stratified columns where both upper and lower layers were normoxic (~85% saturation, ~7.5 mg/L). The vast majority of *L. vincta* larvae recorded in the videos were concentrated at the surface in our experiments. Trends among the small fraction of larvae not clustered at the top 20 mm of the columns showed: (1) slower swimming speed along the path of travel in the hypoxic lower layer than the control lower layer, (2) slower downward velocity in the hypoxic columns than the control columns, (3) faster upward velocity in the hypoxic columns than the control columns, (4) the presence of larvae on the very bottom of the control columns versus a near absence of larvae at the bottom of the hypoxic treatment columns, and (5) a slightly higher fraction of time spent in the lower layer in the control columns. The small magnitudes of these differences may be due to the small number of larvae that traveled down below the surface in the columns in all treatments, or may indicate a relatively low sensitivity to hypoxia in this species. As global climate change continues and coastal hypoxia increases in frequency and severity, it is crucial to improve our understanding of the impacts of hypoxia across ontogenetic stages of marine invertebrates, so as to understand the implications for survival, recruitment, and population resilience. Results of this study demonstrate that upswimming tendencies may reduce encounters of *L.vincta* larvae with hypoxia, that larvae alter swimming behaviors in response to hypoxia, and that the observed behavioral changes appear generally consistent with a weak ability to escape or avoid hypoxia layers.

INTRODUCTION

Hypoxic zones are expanding in coastal waters and the open ocean due to anthropogenic eutrophication and ocean warming (Gilbert et al. 2010, Rabalais et al. 2010). These conditions have various negative impacts on benthic organisms, including decreased species richness, abundance, and biomass (Diaz and Rosenberg 1995). Hypoxia is usually defined as oxygen levels below 2 mg/L (Vaquer-Sunyer and Duarte, 2018), which routinely occur near the benthos in protected ecosystems such as fjords, estuaries, and coastal zones (Diaz and Rosenberg 1995). Gradients in oxygen can be rather sharp near the benthos, and vary in microhabitats and over small time scales (Diaz and Rosenberg 1995). Benthic invertebrates are likely to encounter increasing levels and frequency of deoxygenation at various stages of their life cycles, making it crucial to understand impacts across ontogenetic stages.

A rich literature has focused on the tolerance and behavioral response of adult animals to hypoxia (Modig and Ólafsson 1998, Steckbauer et al. 2015, Galic 2019). However, most marine benthic invertebrates have a larval stage that enables dispersal to new habitats and maintains population connectivity across distances. The ubiquity and important function of larval life stages make it important to quantify the effects of hypoxia on larvae, and understand the ramifications of such effects on benthic populations. Exposure of larvae to hypoxia caused latent effects on juvenile growth of the gastropod *Crepidula onyx* (Li and Chiu, 2013). Reduced survival, delayed metamorphosis, and altered morphology and swimming behavior were observed for larvae of *Nassarius festivus* (intertidal gastropod) reared under hypoxic conditions (Chan et al. 2008). Such direct effects on larval performance and growth indicate that behaviors to avoid hypoxia in the water column may be advantageous to larval populations.

Despite the potential for hypoxia to affect larval stages of marine invertebrates, few studies have investigated the effect of hypoxia on larval behavior specifically. Larval behavior is important for controlling survival in the plankton, dispersal, and eventual settlement. Hypoxia may impose metabolic stress and slow important functions linked to swimming, such as feeding and predator avoidance. The ability to recognize and avoid hypoxic layers may increase survival. Alternatively, larvae may be tolerant to hypoxia levels found in nature, making larval stages comparatively resilient. Understanding the ability of larvae to actively avoid hypoxic layers and how hypoxia influences swimming are important for understanding the survival of larvae in the

plankton and eventual impacts on adult benthic communities in the face of more frequent and intense deoxygenation.

We explored these relationships using pre-competent veliger larvae of *Lacuna vincta. L. vincta* is an abundant small gastropod grazer found on subtidal algae in the Pacific Northwest, Western Atlantic, and Europe. These grazers impact the biomass and abundance of the plant and algal species on which they feed, and indirectly have impacts on whole ecosystem function and nutrient cycling (Krumhansl & Scheibling 2011, Chenelot and Konar 2007). The influence of *L. vincta* on their ecosystem makes it important to understand how their population may be affected by the increasingly hypoxic conditions observed in the northeast Pacific. *L. vincta* produces donut-shaped egg capsules, each containing 1000-1500 eggs, which hatch into planktotrophic veligers after 7-9 days (Strathmann 1987). The larvae become competent to settle after around 60 days in the plankton (Martel & Chia 1991), implying that this species has a prolonged potential to encounter environmental stressors such as hypoxia in the water column. Behavioral responses to avoid such stressors during this long planktonic stage have the potential to significantly impact survival rates and dispersal.

This study has two goals: first, to understand how the swimming behavior of veliger larvae differs in hypoxic versus well oxygenated water; and second to understand how veliger larvae react to encountering a layer of hypoxia in the water column. We conducted video analysis of larvae swimming in stratified water columns, with a layer of oxygenated water overlaying a layer of deoxygenated water. We hypothesized that larvae would behave differently in low DO. More specifically, we hypothesized that, if oxygen limitation slows metabolism, swimming speed in the direction of travel will be slowed. We also hypothesized that, if larvae exhibit hypoxia avoidance responses, they may display relatively higher upward swimming velocities in or near hypoxic layers. Finally, we hypothesized that larvae would avoid deoxygenated water by spending a greater proportion of time spent swimming above the deoxygenated layer relative to fully oxygenated control column, and by exhibiting avoidance behaviors at the hypoxic/normoxic interface, such as reversing direction upon encounter.

METHODS

Larval culture

Lacuna vincta egg cases were collected from algae on the floating dock at Friday Harbor Laboratories (FHL) on August 8, 2019. Egg cases, affixed to the small piece of algae on which they were found, were kept in 800 mL glass jars containing 500 mL of seawater filtered through a fine-meshed filter bag. The jars were continuously stirred by a motorized "paddle set-up" set with a paddle frequency of approximately 10 rpm. Hatching from the egg cases was observed beginning on August 9, 2019. Larvae were fed every other day with *Isochrysis galbana* at a total food concentration in culture jars of ~50,000 cells/mL. Water was changed every other day by reverse filtering through 15 μm mesh down to ~100 mL and then refilling to 500 mL with bag filtered seawater. Larvae were used in trials 6-8 days after hatching.

Seawater preparation for columns

For each trial, 500 mL of bag-filtered seawater was bubbled with nitrogen for ~8 minutes in an Erlenmeyer flask. Dissolved oxygen measurement was taken just before the introduction of this water into the column as well as after each of the trials were run. Control water was bag-filtered seawater at the ambient dissolved oxygen level. To make the upper layer in all columns, "light" seawater was prepared by combining bag-filtered seawater and fresh water in a 15:1 ratio, while the "heavy" lower layer in columns contained undiluted bag-filtered seawater. The salinity difference between upper and lower layers was approximately 2 psu.

Experimental set-up

Four replicate water columns surrounded by a water jacket were used for experiments in a cold-room held at ~12 °C (Figure 1). For each trial, two columns were control treatments and two had a bottom water layer with low DO levels. The columns used for control and those used for low DO were alternated between each trial. The water columns were set up to contain an upper and a bottom layer of water, separated by a halocline. The columns each had dimensions of 350x40x40 mm and were initially filled to approximately 80 mm by pouring in the "light" seawater that was dyed with blue food coloring. Then the "heavy" water for each treatment (either low DO or control water) was introduced to the bottom of the columns by syringe to minimize air exposure, bringing the total water level in the columns to ~155 mm. Larvae were introduced ~30 mm below the surface of each column using a syringe. Between in ~2-3 mL of larvae in "light" seawater were added to each column, with target larval concentrations of ~80

larvae per column. Columns were illuminated by four infrared light sources, two on each side of the columns.

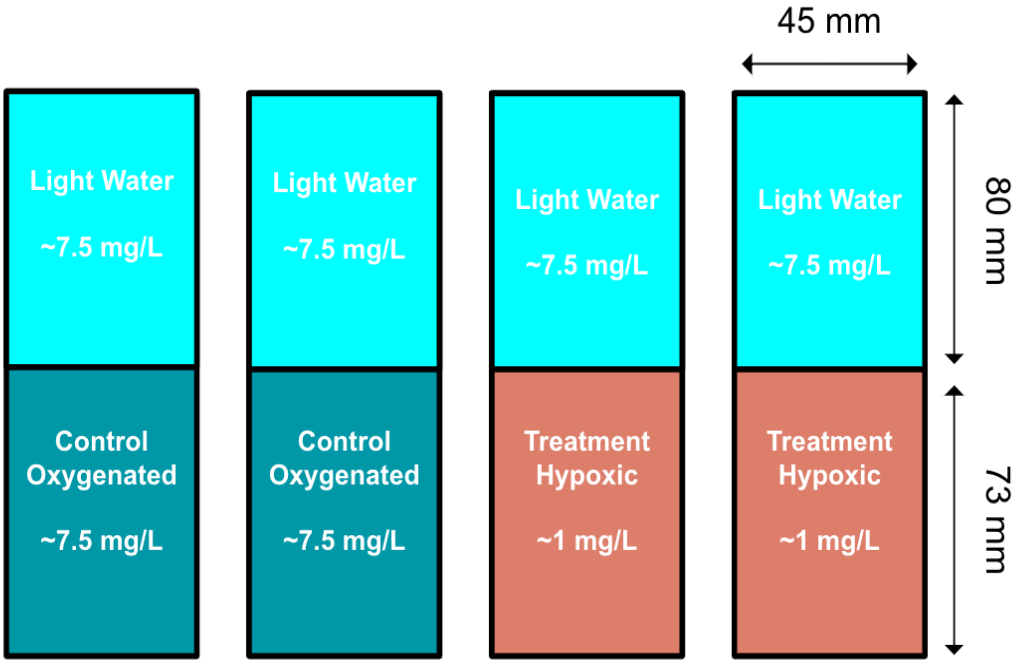


Figure 1. Diagram of water column setup and DO levels used.

Dissolved oxygen measurements

Dissolved oxygen measurements were taken with a YSI proDO meter. Readings were taken from the "light" seawater, control seawater, and low DO seawater before they were added to the columns. After the video collection was completed, the YSI was placed into each column to measure the bottom layer, and the top "light" layer was measured in two columns (on control and one low DO). This post-trial DO measurement was to assess how much oxygen levels in the water changed as the water was added to the columns.

Video collection

Video footage was collected using two cameras (with megapixel 8 mm IR ½" lenses) at frame rate of approximately 29 fps. Fifteen minutes after the last larvae were added, 8622 frames (approximately 5 minutes) of video footage was collected. During video capture, the columns were illuminated by 4 infrared lights, 2 on each side of the experimental set-up, mounted on vertical stands. Lights in the room were off for the 15-minute acclimation period and the period of video recording. The fan to the cold room was turned off for the duration of the recording to avoid water movement from air currents. Videos were recorded using Fosica software

(Wallingford Imaging Systems, Version 0.1001.1027). Seven trials were run, each with two control columns and two hypoxic columns.

Video calibration and analyses

Video was processed using Fosica software. The footage was manipulated to isolate moving particles, which were then exported as position files providing a pixel location for each moving object in each frame of the video. Measurements of the water column were taken to calibrate pixels to true dimensions of the video using the Matlab software package Tracker3D (Grünbaum 2004). Larval positions in each frame were used to create "tracks" of each larva's movement through time for frames 2000-4000 of each video. Swimming velocity vectors were extracted to calculate the speed and direction of larval movement as well as the time spent swimming in different directions. The vertical distribution of larvae in the water columns was also analysed using the same software package.

Statistical analyses

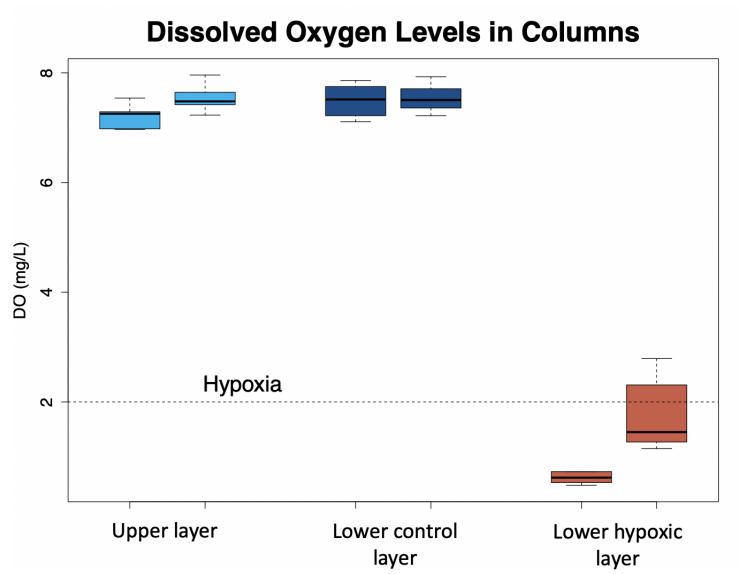
A MatLab program provided by Danny Grünbaum was used to export point locations for larval paths during each frame of the video. It was also used to calculate mean upwards and downward swimming speed and mean total speed. The mean upward swimming speed is the mean upward swimming speed averaged over each tracked swimming path. Mean total speed is the speed in the direction of swimming averaged over each tracked swimming path.

RESULTS

Dissolved Oxygen Levels in the Water Column

Dissolved oxygen was tested before water was added to the columns and after the end of the trial (Figure 2). Before filling the columns, the lowest initial DO level in the treatment water was 0.48 mg/L, and the highest DO level in the treatment water was 0.73 mg/L. No pre-trial data were available for trial 1. At the end of the trials, the lowest DO value in the bottom of the treatment column was 1.15 mg/L and the highest was 2.79 mg/L. Oxygenated light water and oxygenated heavy water did not change in DO values significantly from the beginning to the end of the trial. Treatment DO levels remained below the threshold of what was considered hypoxic (2 mg/L) in all trials except both treatment columns from trials 1 and 2 (final DO levels of 2.79 mg/L, 2.77 mg/L, 2.31 mg/L, and 2.4 mg/L). Water temperature at the start of trials varied from

12.0 °C to 13.9 °C. At the end of trials, temperatures had a much smaller range, varying from 12.2 °C to 12.9 °C.



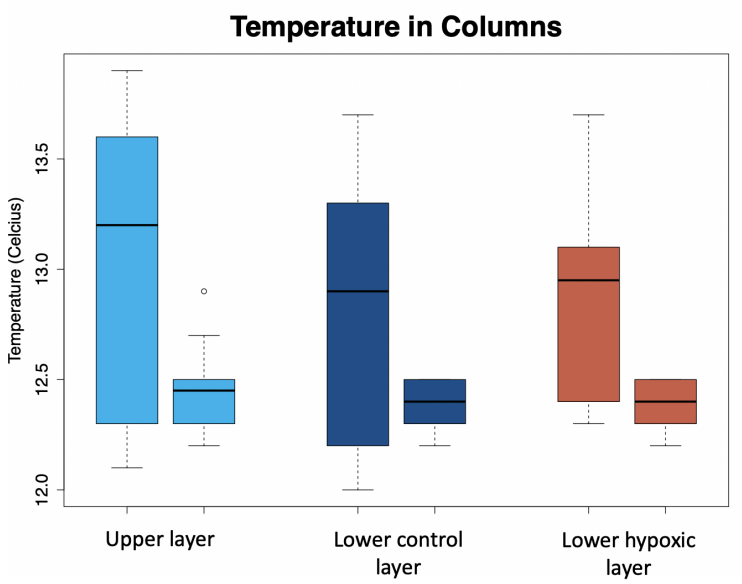


Figure 2. A) Average dissolved oxygen (mg/L) in water used to fill columns taken before water was added to the columns and again after the trial had ended. B) Average temperature (°C) in water used to fill columns taken before water was added to the columns and again after the trial had ended.

Swimming Speeds

Mean upward swimming velocity, mean speed along the path of travel, and mean downward swimming velocity were compared for both layers of each individual column in the study. Over all seven trials, mean upward swimming velocity was slightly slower in both upper

and lower control layers, and slightly faster in both treatment layers (two-way ANOVA, f = 0.42, p = 0.52) (Figure 3a). Swimming speed along the path of travel was consistent between both control and treatment top layers, but slightly slower in the hypoxic bottom layer than the control bottom layer (two-way ANOVA, f = 0.71, p = 0.40) (Figure 3b). Mean downward swimming velocity was greater in both control layers than in both hypoxic layers (two-way ANOVA, f = 2.36, p = 0.13) (Figure 3c). An examination of swimming data in which the seven trials were plotted separately revealed additional insights about behavioral responses. There is much less variation in swimming velocities between the two replicate columns for the top layers, while the swimming velocities exhibited stronger variation between treatment and control bottom layers of replicate columns in each trial. In addition, the extreme ranges of swimming velocities across layers was reflected in large standard deviations, demonstrating diverse larval behavior even within water masses (Appendix Figures 1, 2, 3).

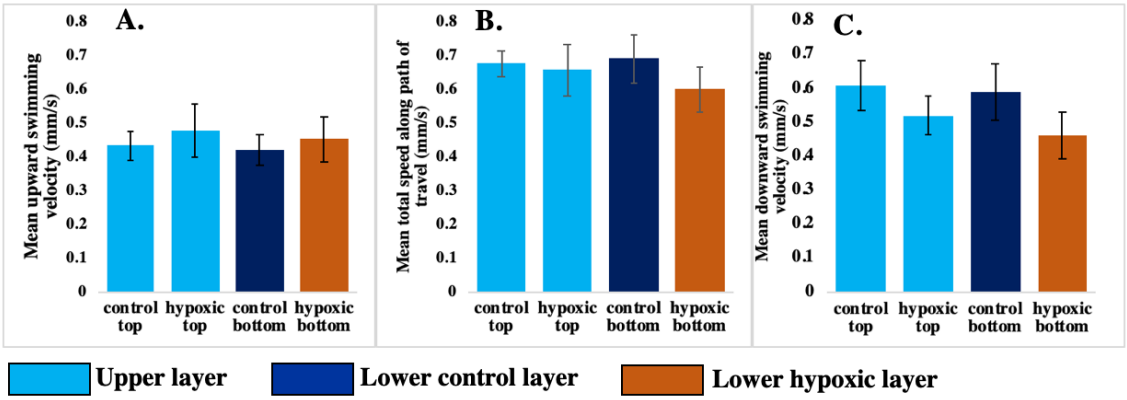


Figure 3. A) Mean upward swimming velocity, mean downward swimming velocity, and mean total swimming speed averaged across all trials for each column quadrant (top layer from control columns, top layer from hypoxic columns, bottom layer from control columns, and bottom layer from hypoxic columns). The 60mm above the layer boundary and 60mm below the layer boundary were used for this analysis, eliminating the upper and lower boundaries of the column. Error bars show +/-1SE.

Time Spent in Different Positions in the Water Column

In both control and treatment columns, larvae concentrated at the surface of the column (Figure 4, top row). Larval density declined towards the bottom of the column; however, a noticeable increase occurred in both control and treatment columns at the halocline for both control and treatment. Larvae were observed at the very bottom of the control column, but not the treatment column. When the top 20 mm were excluded to obtain higher resolution of bottom layers (Figure 4, bottom row), patterns of larval distribution in control and treatment columns

were similar, but differences were easier to identify. The control had a greater number of identified particles than the treatment (Figure 4, note different axis scales).

When observing the full columns, it appears larvae spent a similar amount of time in the top and bottom of the column (cutoff defined as the halocline at 73 mm) between control and treatment (Figure 5a). However, when cluster of larvae in the top 20 mm are removed from the distributions, it is clearer that larvae spent a greater proportion of time in the top of the column for the treatment than the control (Figure 5b).

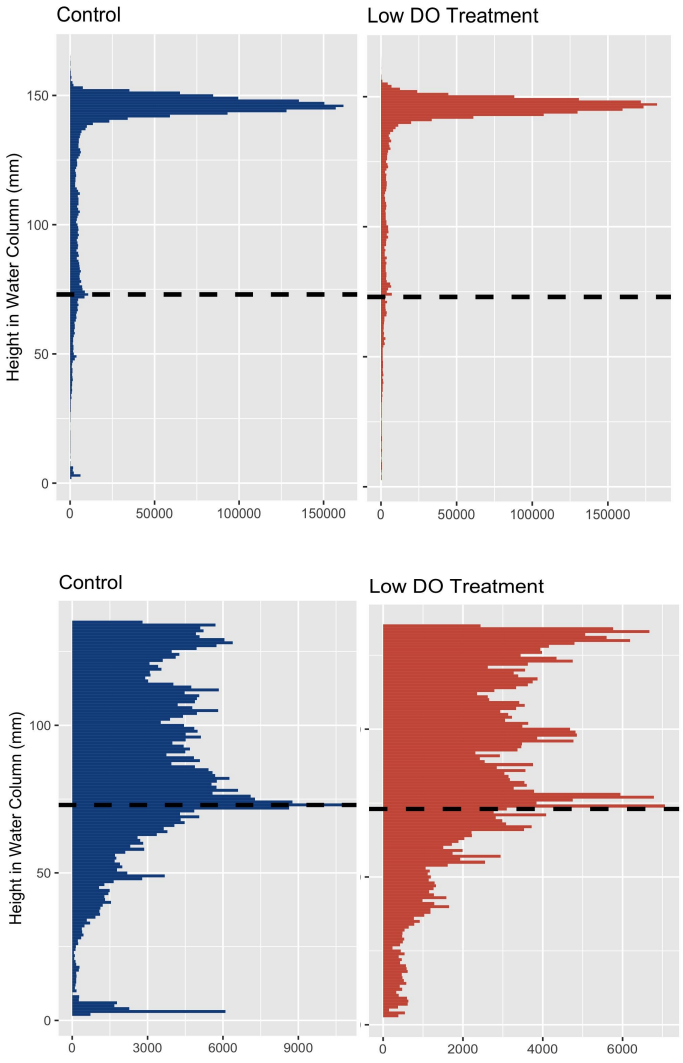


Figure 4. Histograms of time spent swimming at different levels of the water column. Control columns are shown in blue, while treatment columns are shown in red. The top two figures show distributions of the whole column, including the surface layer. The bottom two columns only display data up to 135 mm in the water column, excluding the peak in the top 20 mm.

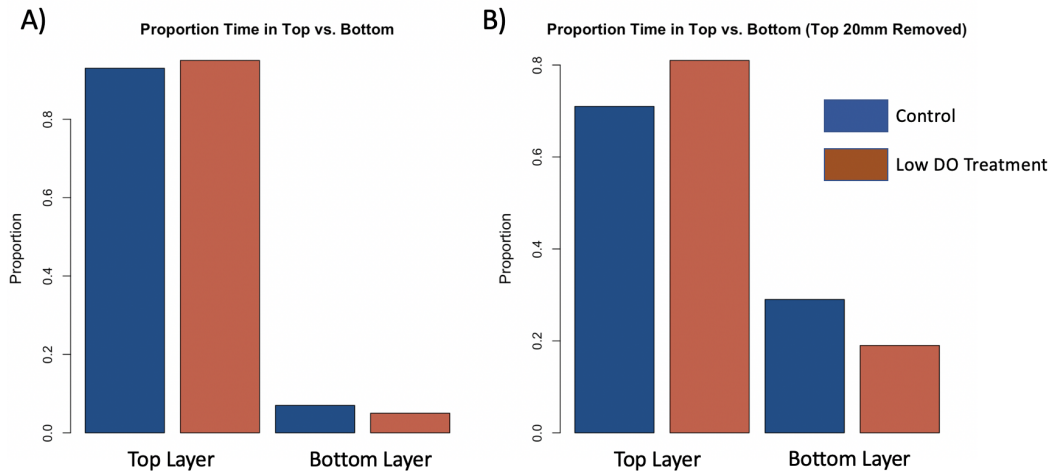


Figure 5. Bar plots of time spent swimming in the top versus the bottom of the water column, with data shown separately for the entire column (A) and the column up to 135 mm, excluding the top 20 mm (B). The leftmost two bars are the top layer and the rightmost two bars are the bottom layer. Control data are shown in blue and treatment data are shown in red.

Larval swimming patterns

Larvae were seen swimming in both upper and lower layers in all columns during all trials. Calculated swimming trajectories indicate that larvae may swim in straight lines as well as in helices. Indications of altered swimming patterns when encountering interface between upper- and lower layers were seen in some cases. Figure 6 presents an example of swimming trajectories calculated from video captured during trial 6. Although many larvae did ultimately cross the halocline interface, this suggests that larvae could detect salinity and/or dissolved oxygen differences across layers, and that they responded with altered swimming to those differences.

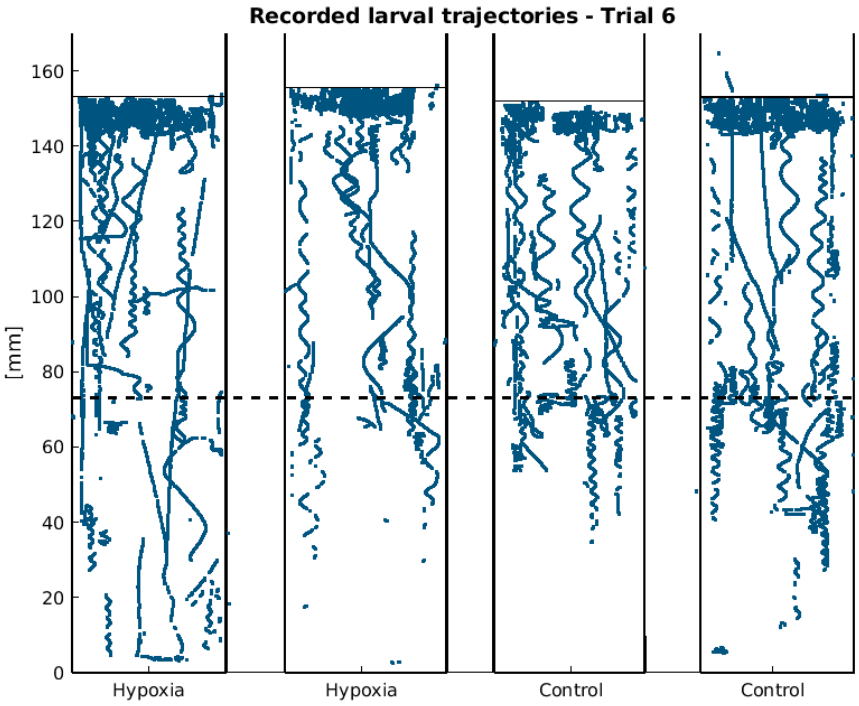


Figure 6. Example of larval swimming trajectories from trial 6. Swimming trajectories are shown in blue while the dashed horizontal line marks the vertical level of the interface between upper and lower layers.

Estimates of Larval Swimming Distances Covered

To assess the effect of small differences in swimming speed over longer time scales, we estimated the differences in vertical positions of larvae assuming the mean observed swimming velocities were maintained for 1 min, 5 min, and 60 minutes (Tables 1, 2, 3). To ensure that the larvae we filmed in our study represented a "steady-state" system, as opposed to a transient period where larvae would still be encountering parts of the columns for the first time, we estimated the time to swim a vertical distance of 16 cm (the height of the water column used in this experiment). Additionally, we extrapolated to estimate the time it would take larvae to swim the height of a 1 m water column and a 10 m water column (Tables 4 and 5).

Table 1. Distance travelled at mean speed along the path of travel (m)

	1min	5min	60min
control top	0.04	0.20	2.43
hypoxic top	0.04	0.20	2.36
control bottom	0.04	0.21	2.48
hypoxic bottom	0.04	0.18	2.15

Table 2. Distance travelled at mean upward velocity (m)

control top	0.03	0.13	1.55
hypoxic top	0.03	0.14	1.71
control bottom	0.03	0.13	1.51
hypoxic bottom	0.03	0.14	1.63

Table 3. Distance travelled at mean downward velocity (m)

	1 min	5 min	60 min
control top	0.04	0.18	2.19
hypoxic top	0.03	0.16	1.87
control bottom	0.04	0.18	2.13
hypoxic bottom	0.03	0.14	1.66

Table 4. Time to swim up the height of the column (min)

	16 cm	1 m	10 m
control top	0.6	3.9	38.7
hypoxic top	0.6	3.5	35.0
control bottom	0.6	4.0	39.8
hypoxic bottom	0.6	3.7	36.9

Table 5. Time to swim down the height of the column (min)

	16cm	1m	10m
control top	0.4	2.7	27.3
hypoxic top	0.5	3.2	32.0
control bottom	0.5	2.8	28.2
hypoxic bottom	0.6	3.6	36.1

DISCUSSION

This study provides the first investigation of *Lacuna* larval swimming behavior in response to a deoxygenation layer in the water column. Most studies on hypoxia in marine environments focus on the survivorship, metabolic performance, and biodiversity of adult animals. The ability of larvae to respond to deoxygenation is a critical knowledge gap and has important implications for survival, dispersal, and recruitment.

We predicted that larvae would show different swimming behavior in hypoxic versus oxygenated water. We expected swimming to slow in hypoxic water due to its impact on overall metabolism, which would potentially decrease swimming competence and feeding rates. Alternatively, we hypothesized that larvae may show a “panic” response to encountering the

deoxygenation layer, and swim upward with greater speed to escape. Speed along the path of travel was relatively consistent across layers and treatments (control top: 0.66 m/s, treatment top 0.66 m/s, control bottom 0.69 m/s), but was slightly slower in the hypoxic bottom layers (0.60 m/s), indicating a possible effect of the hypoxic layer on slowing larval swimming.

We also separately compared swimming velocity in the upward and downward directions to see if larvae tended to move faster in one direction or the other when they were in hypoxic water. Larval downward velocities were slightly slower in both top and bottom layers of the hypoxic columns compared to the control columns (0.52 m/s and 0.46 m/s versus 0.61 m/s and 0.59 m/s), and larval upward swimming velocities were slightly faster in both layers of the hypoxic columns compared to the control columns (0.48 m/s and 0.45 m/s versus 0.43 m/s and 0.42 m/s). The tendency of larvae to slightly slow downward swimming in the hypoxic column may indicate an attempt to minimize their descent into the hypoxic layer. Although comparisons of swimming velocities in hypoxic treatment versus control were not statistically significant, the small observed differences in swimming speeds could have ecological implications on longer time scales. When mean swimming speeds were extrapolated to estimate distances that larvae would travel in one hour, the slight observed differences in velocities manifested in potentially consequential differences in distance covered (2.13 m/hr versus 1.66 m/hr downward swimming for hypoxic versus control bottom water). It is possible that multiple environmental conditions such as food availability, currents, and exposure to cues could vary substantially over meter-scale spatial differences. If larvae in hypoxic versus normoxic waters end up separated by distances at such scales, the other factors they experience in the planktonic phase could vary substantially.

The second hypothesis investigated in this study is that larvae will show avoidance behavior when encountering a deoxygenated layer. The ability to avoid deoxygenation may confer additional resilience to populations in the plankton, as such behaviors may decrease mortality. It may also decrease settlement in benthic locations that experience frequent deoxygenation, which will assure greater survivorship after colonization. Alternatively, if larvae avoid settlement due to deoxygenation near the benthos, recruitment may be hindered. To test this hypothesis, we compared the distribution of larvae in the water column between control and hypoxic columns. Distributions were largely dominated by the large number of larvae in the top centimeter of the columns. When this area was excluded from the histograms, very slight trends

could be observed: larvae in both the control and hypoxic columns appeared to react to the halocline, as indicated by the peak in tracked points around the interface in both treatments. More larvae spent time on the very bottom of the column in the control treatment, indicating that larvae in the hypoxic column may have avoided staying on the very bottom of the column. In addition, when the top 20 mm were excluded, the fraction of time spent in the bottom layer was slightly lower for the hypoxic treatments than the control. Both treatments showed a decrease in tracked particles with increasing depth into the lower layer. Altered swimming patterns when encountering the interface between upper- and lower layers were observed for some larvae and corresponds to the peak in distribution seen in Figure 7. Swimming helices tend to become tighter and often more compressed along their central axis in the vicinity of the interface.

Overall, the larvae in our study showed a clear preference for remaining very close to the surface of the columns in both control and hypoxia treatments (Figure 9). Preliminary observations had revealed this preference for remaining near the surface. However, a previous study by the authors demonstrated that a 20 minute acclimation time in the columns in the absence of light was sufficient for larvae to begin exploring throughout the column and reach a more even distribution. In contrast, the data in this study were dominated by the vast majority of larvae convening at the tops of the column. It is unclear if the larvae explored the full length of the water column in the 20-minute acclimation period, or whether they remained near the surface since introduction and therefore never experienced the different water layers. If this dominant upward swimming behavior holds true in field settings, it has important implications for larval dispersal, potential larval exposure to hypoxia, and exposure of possible settlement cues. In addition, this information is valuable for predicting larval distributions in the field, inferring dispersal patterns from surface water dynamics, and planning plankton tow collections of *L. vincta* larvae.

While our data were largely dominated by surface swimming behaviors, there are small trends indicating an avoidance response to hypoxia. These small differences could be due to the relatively few number of larvae that ventured below the surface layer and therefore a small sample size of larvae in the vast majority of the column. However, these small differences between treatments may indicate a lower sensitivity to low DO for larvae of *L. vincta*, at least over short time scales. In preliminary experiments in flasks before these video analyses, *L. vincta* larvae continued to swim in deoxygenated water for hours, and while they appeared to amass at

the surface, this was also noted in flasks of control water, and appears to be a common behavior in small vessels. It has been noted before that some taxa are more sensitive than others, and mollusks are relatively robust to low oxygen (Vaquer-Sunyer and Duarte 2008), which may be true for *L. vincta*. Our study was focused on short term encounters, so future studies should examine whether there are impacts on larval behavior over longer-term exposures. If larvae are in fact harmed by longer exposures to low DO, the observed minimal avoidance behavior may have consequences for survival in the field.

CONCLUSION

Hypoxia has the potential to influence marine organism function at many stages in the life cycle. Most studies on benthic invertebrates have focused on adult animals, however impacts on larvae will have important consequences for survival and recruitment of benthic populations. We sought to answer whether hypoxia changes the swimming behavior of gastropod veliger larvae, and whether larvae are able to recognize and avoid hypoxic layers. The vast majority of *L. vincta* larvae were concentrated at the surface in all columns of our experiments. In addition, small trends were noted between control and low DO treatment: 1) slower swimming speed in the lower hypoxic layers than the control layers, 2) decreased downward velocity and increased upward velocity in the hypoxic columns than control columns, and 3) a higher percentage of time spent in the upper layer for larvae in columns containing a hypoxic lower layer. Additionally, the presence of larvae on the very bottom of the column in the control that was not noted in the hypoxic treatment. The small magnitude of these differences may be due to the small number of larvae that traveled down in the columns in all treatments, or may indicate a relatively low sensitivity to hypoxia by this species. The observed decrease in swimming speed along the path of travel in the hypoxic water should be investigated further, as it may influence larval ability to control position in the water column and may impact feeding rates. As hypoxic and anoxic "dead zones" are projected to increase in extent in the oceans, especially along coasts, it is crucial to improve our understanding of the impacts of hypoxia on various stages of marine animal life cycles, as these have implications for survival, recruitment, and population resilience.

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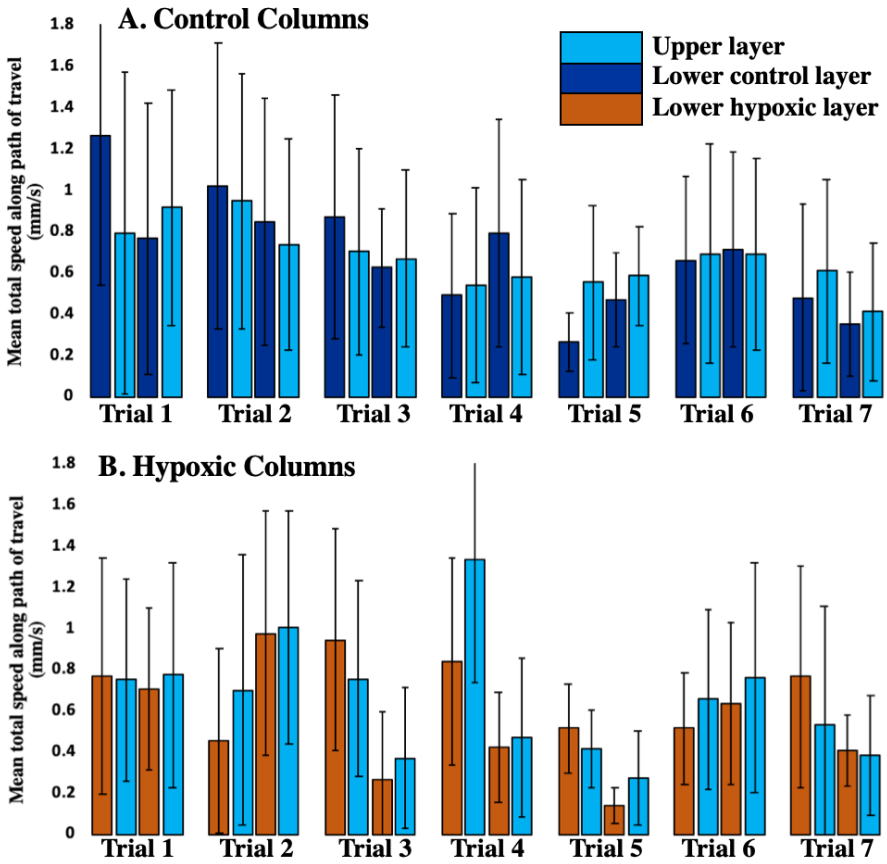
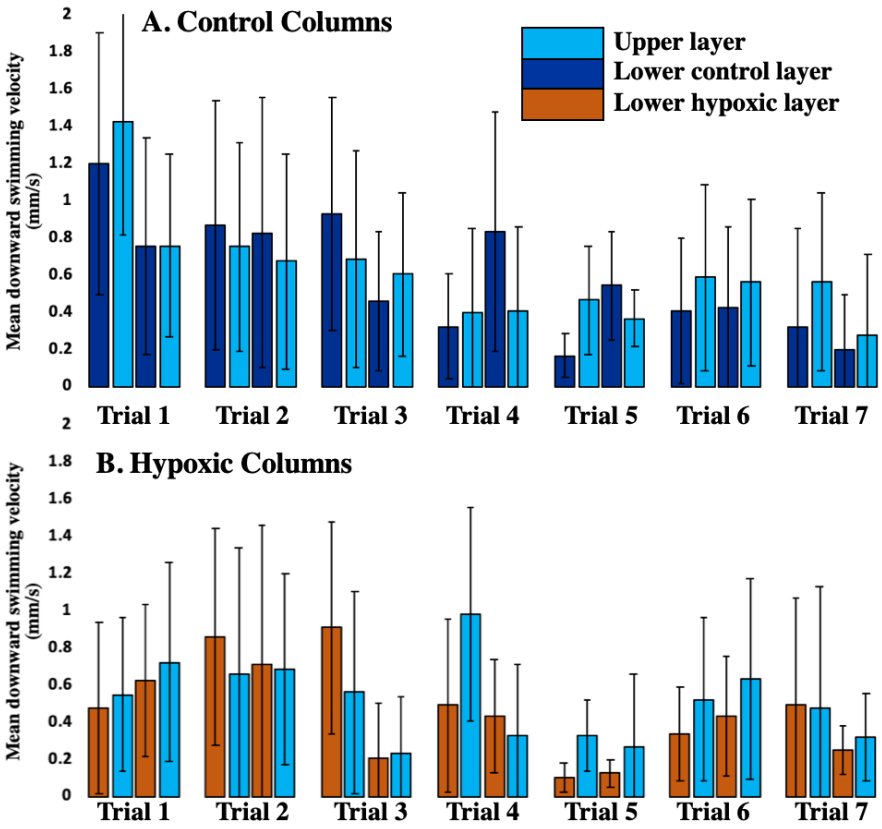
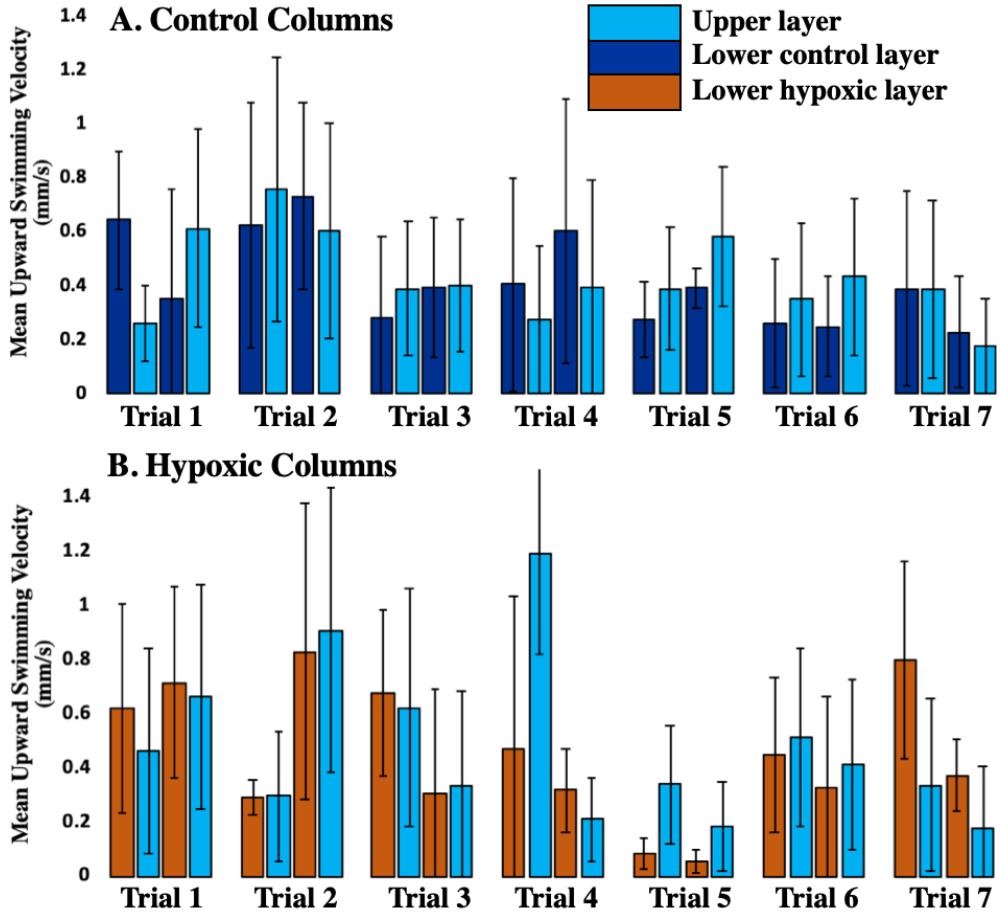
APPENDIX



Appendix Figure 1. Mean total speed along the path of travel for larvae in the top and bottom layer of each column type during each of the 7 trials. There were two control columns and two columns with hypoxic bottom layers in each trial. The 60mm above the layer boundary and 60mm below the layer boundary were used for this analysis, eliminating the upper and lower boundaries of the column. Error bars show +/-1SD.



Appendix Figure 2. Mean downward swimming velocity for larvae in the top and bottom layer of each column type during each of the 7 trials. There were two control columns and two columns with hypoxic bottom layers in each trial. The 60mm above the layer boundary and 60mm below the layer boundary were used for this analysis, eliminating the upper and lower boundaries of the column. Error bars show +/-1SD.



Appendix Figure 3. Mean upward swimming velocity for larvae in the top and bottom layer of each column type during each of the 7 trials. There were two control columns and two columns with hypoxic bottom layers in each trial. The 60mm above the layer boundary and 60mm below the layer boundary were used for this analysis, eliminating the upper and lower boundaries of the column. Error bars show +/-1SD.