Changes in Larval Oyster Swimming Behavior with Salinity and Larval Age

Emily C. Manuel[1], Joseph Caracappa[2], and Daphne Munroe[1,*]

[1] Haskin Shellfish Research Laboratory, Rutgers University, 6959 Miller Avenue, Port Norris, New Jersey 08349
[2] NOAA Northeast Fisheries Science Center, 166 Water Street, Woods Hole, Massachusetts 02543

Abstract

*Eastern oysters (*Crassostrea virginica*) are sessile, relying on a larval phase to disperse in estuaries. Oyster larval swimming behavior can alter dispersal trajectories and patterns of population connectivity. Experiments were conducted to test how both (1) acclimation time to new environmental conditions and (2) larval swimming behavior change with salinity and larval age. Acclimation time to changes in salinity was longest in lower salinity (6 ppt) and decreased with age. To test changes in behavior with salinity, larvae were placed into four salinities (6, 10, 16, and 22 ppt) where swimming was recorded. To test changes in behavior with age, larvae aged 6, 12, and 15 days were recorded. In both experiments, swimming paths were mapped in two dimensions, behavior of each path was categorized, and speed, direction, and acceleration were calculated. The frequency of upward, neutral, and downward swimming behaviors did not differ across salinity treatments but did vary with age, whereas the frequency of behavior types varied with both salinity and ontogeny. As an example, diving was observed more frequently in low salinity, and more downward helices were observed in moderate salinity, while younger larvae swam upward with more frequency than older larvae. Surprisingly, diving was observed in 10%–15% of all larvae across all ages. Given the consequence of larval behavior to marine invertebrate dispersal, changes in swimming over larval age and in response to environmental changes have important implications to marine population stability and structure.*

Introduction

Estuaries are the dynamic interface between rivers and the ocean. This gradient from fresh water to the salty ocean creates habitat for eastern oysters (*Crassostrea virginica*), and due to their phenotypic plasticity, oysters are able to compensate for the unique challenges estuaries present (Eierman and Hare, 2013; Guo *et al.*, 2018). Oysters are euryhaline and can tolerate salinities ranging from 5 to 35 ppt (Galtsoff, 1964), but their optimal range is 14 to 28 ppt (Shumway, 1996), which makes estuaries suitable habitat. Oysters build reproductive capacity starting in spring and spawn throughout the summer and into the fall if conditions remain favorable (Galtsoff, 1964). After spawning and fertilization, oyster larvae continue to develop in the water column during a 2- to 3-week planktonic larval period (Galtsoff, 1964), which allows for the natural redistribution of individual oysters (Haskin, 1964; Dekshenieks *et al.*, 1996; Narváez *et al.*, 2012).

Oyster larvae have historically been modeled as passive particles and were considered to be transported solely *via* physical processes (Thorson, 1950; Korringa, 1952; Verwey, 1966). Over time, additional studies have found that larval swimming patterns respond to environmental factors such as salinity (Haskin, 1964; Hidu and Haskin, 1978; Mann *et al.*, 1991), turbulence (Fuchs *et al.*, 2013, 2015), light (Wheeler *et al.*, 2017), and food availability (Maciejewski *et al.*, 2019). Oyster larvae generally exhibit one of three swimming behaviors: helical swimming, sinking, or diving. Helical swimming is an exploratory behavior, which allows larvae to sense the nearby environment and assess its suitability while minimizing

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* Corresponding author; email: dmunroe@hsrl.rutgers.edu.
Abbreviation: NGDR, net-to-gross distance ratio.
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travel (Maciejewski *et al.*, 2019). Sinking occurs when larvae draw in their velum and passively descend due to their negative buoyancy (Wheeler *et al.*, 2015). In contrast, diving is an active process that requires a propulsive downward acceleration, allowing larvae to penetrate the benthic boundary layer, contact the bottom, attach, and settle (Finelli and Wethey, 2003; Fuchs *et al.*, 2013). Aside from studies documenting changes in the swimming speed of bivalve larvae of varying life stages (Mann and Rainer, 1990; Mann *et al.*, 1991; Troost *et al.*, 2008), little is known about the relative frequency of these swimming behaviors or the ways in which environment and developmental stage (*i.e.*, ontogeny) alter behavioral characteristics.

Oyster larvae in estuarine habitats experience rapid changes in salinity due to tides and acute weather events. Tidally driven salinity differences between the ebbing and flooding tides tend to be moderate in comparison to those driven by acute freshwater events driven by storms (Delaware Bay Salinity OFS Nowcast, 2021). Low-salinity freshets can reduce estuarine salinity for prolonged time periods—several weeks to months (Munroe *et al.*, 2013)—and projections show an increase in the frequency and intensity of storm events due to climate change (Najjar *et al.*, 2000, 2009). The increased frequency of extreme low-salinity events could pose a risk to oyster larval survival if larval swimming changes in response to low salinity such that the ability of larvae to access suitable habitats is compromised. In addition, larval oysters have been observed to swim faster as salinity increases (Hidu and Haskin, 1978) and to decrease swimming activity in low salinity in late-stage larvae (Haskin, 1964). Thus, in low-salinity habitats, larvae would tend to be lower in the water column and thus more likely to be retained in those environments due to tidal stream transport (Haskin, 1964).

Larval swimming has been shown to change with age (Mann and Rainer, 1990; Mann *et al.*, 1991; Troost *et al.*, 2008). However, studies of oyster larval behavior often use competent (late stage, nearing metamorphosis) larvae in laboratory enclosures (Fuchs *et al.*, 2013; Wheeler *et al.*, 2017; Maciejewski *et al.*, 2019). As larvae age, they grow larger and become faster swimmers (Troost *et al.*, 2008), making them better able to regulate their vertical position (Hidu and Haskin, 1978; Mann *et al.*, 1991). Ontogenetic changes in *C. virginica* larval swimming behavior could vary in several ways. Larvae near metamorphosis may exhibit more downward behaviors (downward helices, sinking, and diving), which could increase the likelihood of contact with bottom settlement habitats (Fuchs *et al.*, 2013). Conversely, competent larvae may exhibit more helical behaviors to maintain their location near suitable habitat (Maciejewski *et al.*, 2019).

The aim of this study is to characterize *C. virginica* larval swimming behavior with respect to changes in salinity and larval age. An increase in the magnitude of acute salinity changes is expected to lengthen larval acclimation times and to alter the frequency and characteristics of larval behaviors. Likewise, swimming behavior is expected to change as larvae grow and develop, with faster and more downward swimming expected in older larvae. In this study, experiments were conducted to determine how both (1) acclimation time to new environmental conditions and (2) larval swimming behavior change with salinity and larval age. Ultimately, these experiments will improve understanding of oyster larval swimming and help parameterize future larval dispersal model behaviors.

Materials and Methods

Larval care

Larvae were spawned using adult *Crassostrea virginica* (Gmelin, 1791) oysters obtained from a disease-resistant strain (Guo *et al.*, 2008) crossed with wild Delaware Bay oysters. Adult oysters were sexed and then strip spawned using 21 males and 39 females, with a ~93% fertilization success. After fertilization, all embryos were reared in one culture from which a subsample of larvae were removed (without replacement) at various times for all experiments. Larvae were reared according to standard hatchery protocols (Helm and Bourne, 2004) at 22 ppt. Water changes occurred three times each week, during which larvae were counted and shell measurements of 10 randomly selected larvae were made with a stage micrometer (accurate to ±0.01 μm). Size and growth were calculated prior to each experiment (Table 1).

Acclimation experiment

About 450 larvae were removed from the larval culture and placed into 50-mL conical polyethylene centrifuge tubes (Corning Falcon, Tewksbury, MA) (for larval densities of ~10 mL^{-1}) at four different salinities (6, 10, 16, and 22 ppt) with three replicates per salinity (12 tubes in total). Salinity was measured using a Fisherbrand digital refractometer (Fisher Scientific, Waltham, MA). Acclimation time was defined as the time it took 80 larvae to maintain a vertical position above the 35-mL line on the tubes (~8 cm from the base). This level of larval activity was chosen because it reflected the activity level of larvae that had not been transferred to a new salinity environment. Counts were repeated every 2 h until the acclimation time criteria were met. Larvae were provided food at approximately 20,000 algal cells mL^{-1} of *Isochrisysis galbana* (Tahitian strain) to reduce the effects of starvation on swimming. Acclimation time measurements were repeated at three ages (6, 12, and 15 days).

Salinity experiment

Ten-day-old larvae were placed in aerated chambers at four salinity treatment levels (6, 10, 16, and 22 ppt) for 2 days prior

Table 1

Larval sizes at each *Crassostrea virginica* experimental age (n = 10)

	6 days	12 days	15 days
Mean ± SE (μm)	98.0 ± 2.9	163.0 ± 6.2	264.0 ± 5.2

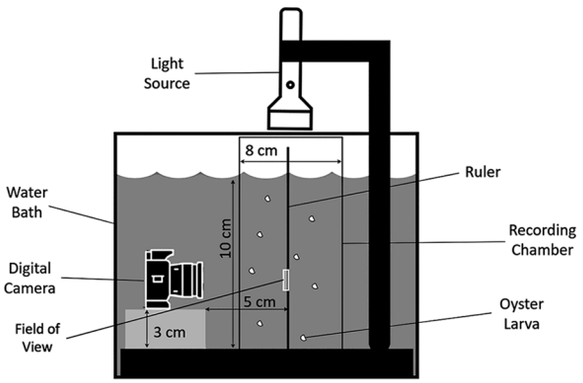

Figure 1. Schematic of the aquarium, lighting, and video setup used to record larvae. Modified from Caracappa and Munroe (2019).

to experiments, allowing them to acclimate to the salinity change. The acclimated larvae were placed into the salinity treatment tank matching the salinity in which they were acclimated (larval size was measured before and after acclimation to ensure that size had not significantly changed). Swimming observations were made in 9-L rectangular tanks with three simultaneous replicates of each salinity treatment (6, 10, 16, and 22 ppt).

Ontogeny experiment

To determine the changes in swimming behavior with age, larvae of three ages (6, 12, and 15 days) were used with a consistent salinity of 10 ppt. While the 15-day-old larvae were the largest (Table 1), they did not have fully developed eye spots and were not yet competent.

Video recordings

Larval swimming behavior was recorded following methods described in Caracappa and Munroe (2019) for both the salinity and ontogeny experiments (Fig. 1). A focused overhead light (color temperature = 7000 ± 1000 K; intensity = 2250 ± 250 mcd) was used to illuminate the larvae, and larvae were recorded for 18 min using an Olympus waterproof digital camera. The camera was submerged in the water bath and elevated to half the height of the recording chamber. The focal area was 1.5 cm high × 2 cm wide, with a focal distance of 5 cm. If no larvae were observed swimming during the 18-min recording, an additional 18-min recording was made with the same larvae. After acclimation, approximately 550 larvae were placed into recording chambers filled with 1300 mL of filtered seawater (~0.5 larvae mL^{-1}). Prior to recording, each chamber (containing larvae) was placed into an ambient-temperature (20 °C) water bath in the dark for 30 min. The water bath served to reduce refraction when recording larval movement in water and to stabilize changes in temperature.

Data analysis

One-way ANOVA was used to test whether acclimation time differed across salinity treatments or age. Videos of larval swimming paths were analyzed in Kinovea, an open-source 2D video analysis software (Charmant, 2019), to calculate horizontal (X) and vertical (Y) positions of each larva (~60 frames per second). The first minute of each video was omitted from analysis as to avoid any motion due to manual adjustments. Larval paths less than 1 cm in gross displacement were removed from the analysis because they were too short to characterize a behavior. The number of tracked swimming paths varied for each replicate depending on how many larvae were active in frame and in focus, with an average of 30 and 37 paths per video in the salinity and ontogeny experiments, respectively (Table 2).

For each larval path, five swimming characteristics were calculated: velocity and acceleration (mean, standard deviation, minimum, and maximum), net-to-gross distance ratio (NGDR), standard deviation of the X position, and swimming angle. Velocity and acceleration are indicators of larval swimming ability and were measured as the mean first and second differences, respectively, of the displacement (X–Y). The first and second differences refer to the incremental change of each time series and are equivalent to the first and second derivatives but for discrete data time series. The standard deviation of X positions was used to differentiate helix behaviors such that helix width increases as the standard deviation of X increases. The NGDR was defined as the ratio between the net Euclidian distance (final position minus initial position) and the gross distance (full length of the traced larval path). Straight paths have an NGDR of 1, while a random path will approach 0.03 (Caracappa and Munroe, 2019). Larval swimming angle (θ) was defined as

$$\theta = \tan^{-1} \frac{Y_{\text{final}} - Y_{\text{initial}}}{X_{\text{final}} - X_{\text{initial}}}$$

such that 0° was horizontally to the right and 180° was horizontally to the left of the video frame. Larvae with $0° < \theta < 180°$ were defined as upward swimming and $180° < \theta < 360°$ as downward swimming (Fuchs *et al.*, 2013, 2015). Due to the small magnitude of vertical displacement, neutral behaviors could not be categorized by net swimming angle but were considered paths with small Y displacements and an NGDR >0.45. A χ^2 test for independence was used to determine whether the frequency of observation of various swimming characteristics (*i.e.*, vertical direction or specific behavior) differed across salinity and age treatments. All statistical analyses were performed in R (R Core Team, 2021).

Table 2

Number of *Crassostrea virginica* larval paths analyzed per video

	Salinity experiment				Ontogeny experiment		
Replicate	6 ppt	10 ppt	16 ppt	22 ppt	6 days	12 days	15 days
A	50	55	34	47	2	55	34
B	42	28	33	19	22	28	48
C	27	44	32	31	19	44	16
Average	40	42	33	32	14	42	33

Swimming behaviors were defined using characteristics of each larval path. A key was created (Fig. 2) showing the relationship of these behavior types with overall path direction. First, all paths were characterized by their predominant vertical (Y) direction (upward, downward, or neutral). Then, the X position was used to determine whether larvae were moving in a straight or oscillating pattern. If the X position oscillated, behavior type was characterized as helices, where the standard deviation in the X position was used to categorize helices as wide (SD > 0.12), moderate (SD < 0.12 and SD > 0.05), or narrow (SD < 0.05) helices (Table S1, available online). Diving was differentiated from straight down (sinking) as tracks that reached a minimum velocity of 0.4 cm s^{-1} and a minimum acceleration of 3.0 cm s^{-2} (following cutoffs in Wheeler *et al.*, 2015). Larval paths sometimes included more than one behavior type. For paths showing more than one type of behavior, the path was assigned the behavior observed at least half the time in the frame. Summary statistics including average velocity, average acceleration, NGDR, variability in the horizontal direction, and angle for each of the behaviors in Figure 2 are provided in Table S1. Behaviors that could not be defined using this key were removed from analysis (salinity: $n = 2$; ontogeny: $n = 3$). The relative frequency of each behavior was calculated and compared between salinity and age treatments using a likelihood ratio χ^2 test.

Results

Average characteristics (velocity, acceleration, NGDR, standard deviation in X, and angle) measured for each behavior are provided in Table S1.

Acclimation experiment

Acclimation time differed with salinity and age (Fig. 3). As salinity decreased, larvae took significantly longer to acclimate ($F = 7.3$, df = 32, $P = 0.01$), and acclimation time decreased significantly as larvae aged ($F = 19.2$, df = 32, $P < 0.001$). There was a significant interaction between salinity and age ($F = 20.8$, df = 30, $P \ll 0.001$) such that as larvae aged, their acclimation to different salinities occurred faster. Indeed, 6-day-old larvae acclimated in 5–32 h, whereas 12-day-old larvae acclimated in 9–12 h for all salinities (Fig. 3). For 15-day-old larvae, acclimation time was immediate at higher salinity (16 and 22 ppt) and 9 h at the two lower-salinity treatments. Six-day-old larvae in the 6-ppt treatment acclimated in only one replicate (Fig. 3).

Salinity experiment

Overall, relative frequency of behavior directions (upward, neutral, downward) did not differ between salinity treatments for larvae of the same age ($\chi^2 = 3.61$, df = 6, 444, $P = 0.73$; Table 3; Fig. 4). The relative frequency of behavior types varied between salinity treatments ($\chi^2 = 73.98$, df = 36, 444, $P < 0.001$), with a higher frequency of diving observed in low salinity (6 ppt), more downward helices observed in 10 ppt, and increased incidence of straight up behaviors in 16 ppt (Fig. 5). Of all larval behaviors observed, 43% swam upward, 41% swam downward, and 16% were vertically neutral (Fig. 4).

Ontogeny experiment

The relative frequency of swimming direction (upward, neutral, downward) differed significantly as larvae aged ($\chi^2 = 15.4$,

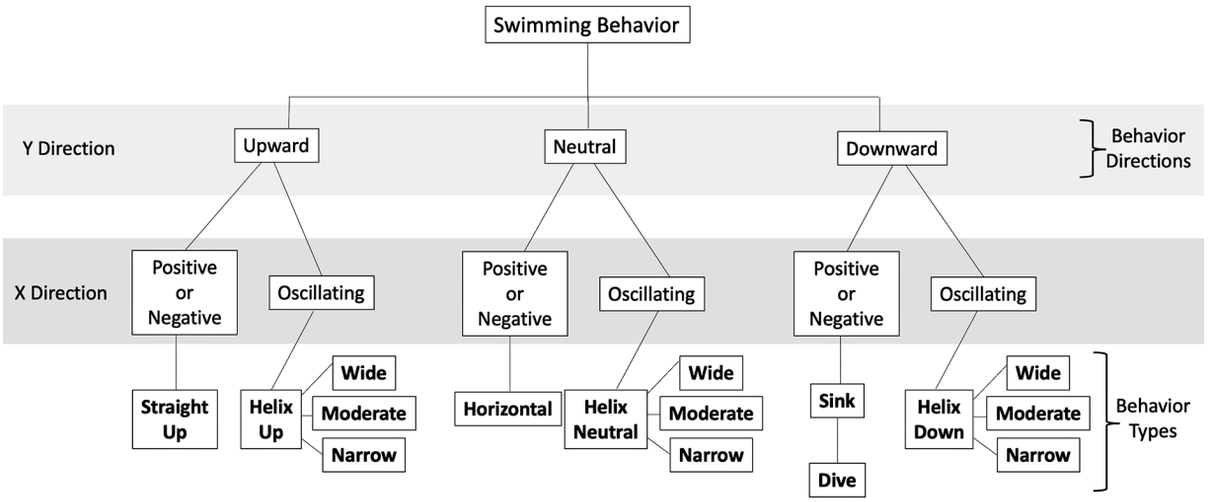

Figure 2. Key used for defining behavior of each *Crassostrea virginica* larval track based on observed horizontal (X) and vertical (Y) positions. Final behaviors in the different directions are shown in bold: upward ($0° < \theta < 180°$), neutral (short distance and net-to-gross distance ratio [NGDR] > 0.45), and downward ($180° < \theta < 360°$). Helix type was differentiated by standard deviation in the X direction (wide: SD > 0.12; moderate: SD < 0.12 and SD > 0.05; narrow: SD < 0.05). Diving was differentiated from straight down (sinking) using minimum velocity (0.4 cm s^{-1}) and minimum acceleration (3.0 cm s^{-1}) reached (following cutoffs in Wheeler *et al.*, 2015). Average swimming characteristics (velocity, acceleration, NGDR, standard deviation in X, and swimming angle) measured for each behavior are provided in Table S1, available online.

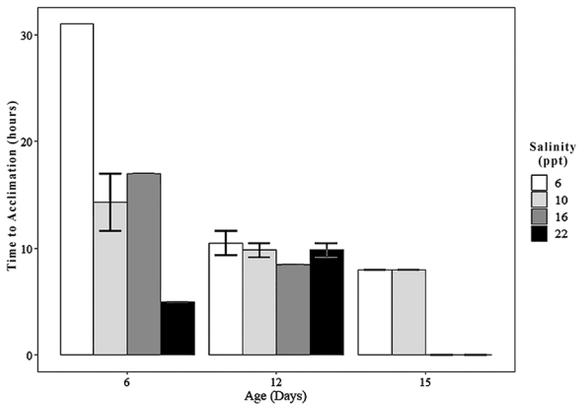

Figure 3. Time to acclimation (mean ± SE) in different salinities (indicated by bar shade) and across ages of *Crassostrea virginica*. No error bars are shown for the 6-ppt treatment at age 6 days because $n = 1$ due to no larval response in two replicate treatments. Larvae in the two highest salinities acclimated began swimming immediately at 15 days old.

df = 4, 268, $P = 0.0038$; Table 3; Fig. 5). Upward behaviors were most frequent in 6-day-old larvae, and 12-day-old larvae swam upward and downward equally (Table 3). Downward swimming increased in frequency as larvae aged, with the oldest larvae (15 days) swimming downward approximately 65% of the time (Table 3; Fig. 4). Similarly, the frequency of behavior types varied with larval age ($\chi^2 = 120.9$, df = 24, 268, $P \ll 0.0001$; Table 3; Fig. 5). Six-day-old larvae showed a higher frequency of narrow helix up, and 15-day-old larvae showed more narrow helix down and fewer narrow helix up (Fig. 5). Diving was observed at all ages, exhibited by 10%–15% of all larvae across all ages (Fig. 5).

Discussion

Experiments were conducted to test how both (1) acclimation time to new environmental conditions and (2) larval swimming behavior change with salinity and larval age. Acclimation time to changes in salinity was longest in lower salinity, and older larvae took less time to acclimate. The frequency of upward, neutral, and downward swimming behaviors observed did not differ across salinity treatments but did vary with age, whereas the frequency of behavior types varied with both salinity and ontogeny. As an example, diving was observed more frequently in low salinity, and more downward helices were observed in moderate salinity, while younger larvae swam upward more frequently than older larvae. Surprisingly, diving was observed in 10%–15% of all larvae across all ages. While straight upward swimming in bivalve larvae is not well documented, in contrast to diving, it may provide a way to rapidly move higher in the water column. The two vertical behaviors in combination (diving and straight upward swimming) may allow for directed tidal transport. Quantitative evaluations of X–Y swimming paths have been analyzed in other organisms such as blue crabs (Caracappa and Munroe, 2019), larval fish (Erftemeijer *et al.*, 2009), and other bivalves (Mann and Rainer, 1990; Mann *et al.*, 1991; Troost *et al.*, 2008) but have not been extensively characterized for early-stage *Crassostrea virginica*. Herein, a broader characterization of swimming behaviors was examined, and the frequency of how often these behaviors are exhibited provides an overall picture of how these larvae behave.

Energy expenditure in an oyster larvae is split between growth, development, and swimming (Fuchs *et al.*, 2017). Different swimming behaviors presumably incur different energetic costs, yet relatively few studies have quantified these costs across behavior types given the challenges of controlling behavior in an experimental setting. Diving may be the costliest behavior observed, because it requires a forceful propulsion downward and exhibits a greater velocity than all other behaviors (Finelli and Wethey, 2003). Otherwise, upward movements are likely costlier than downward behaviors, requiring larvae to move against gravity. Modeling the energetic costs of behaviors is outside the scope of this study, but our measurements and relative frequency of behaviors could be used to inform future studies modeling oyster larval energetics.

Acclimation experiment

Acclimation time after a salinity shock has not been, to our knowledge, previously reported for oyster larvae despite the potential relevance to oyster larvae in the field. Oyster larval swimming has been shown to slow as salinity decreases until cessation of activity in extreme low salinity (Haskin, 1964). Since larvae move with the tide and are not fixed in place, the role of acclimation could be important in realized estuarine dispersal. In the present study, when transferred to lower salinity (a decrease of 12 ppt or more), larvae temporarily stopped swimming but eventually resumed. Acclimation time increased as salinity decreased and decreased as larvae aged. Older larvae have higher tolerance to changing environments (Dove and O'Connor, 2007), which may explain our observed decreased acclimation time as larvae age.

Table 3

Percentage of observed *Crassostrea virginica* behaviors in each vertical direction for each age and each salinity treatment across all replicates

	Upward behaviors (%)	Neutral behaviors (%)	Downward behaviors (%)
10 ppt			
Age (days)			
6	51	10	39
12	43	12	45
15	22	13	65
10 days			
Salinity (ppt)			
6	42	17	41
10	43	12	45
16	48	15	37
22	39	19	42

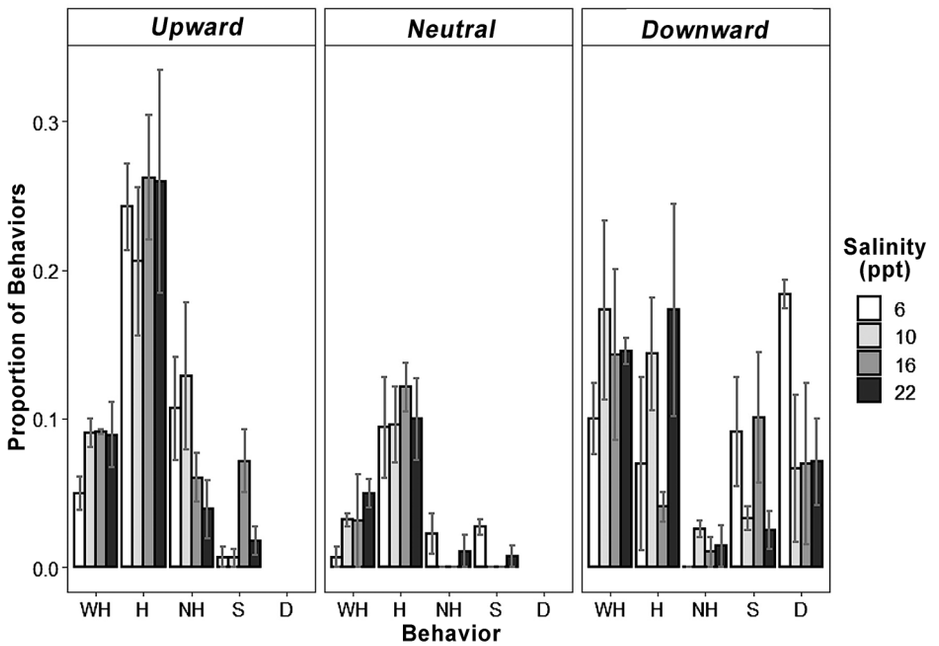

Figure 4. Proportion of behaviors (mean ± SE; $n = 3$) exhibited by *Crassostrea virginica* in different salinity treatments (indicated by shading). Behavior types along the *x*-axis are wide helix (WH), helix (H), narrow helix (NH), straight (S), and diving (D), separated by behavior directions represented by the panels.

Acclimation time may have increased with decreasing salinity because oysters are osmotic conformers, where they adjust the osmolality of their hemolymph to match their external environment (Galtsoff, 1964). Adjusting hemolymph osmolality is energetically costly in low salinity (Freire and Sampaio, 2021), thus potentially reducing available energy for larval swimming. Furthermore, lower-salinity environments could act as an ecological trap, particularly for younger larvae if larvae are unable to acclimate to acute changes in salinity, especially since the frequency of low-salinity freshets is anticipated to increase in the northeast region (Sanderson *et al.*, 2019; Maxwell *et al.*, 2021).

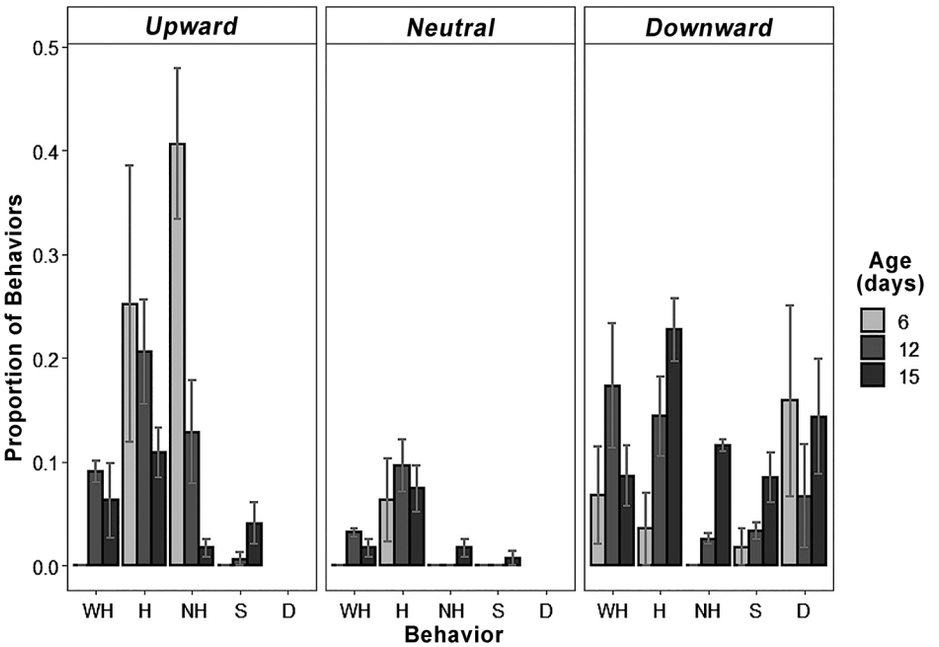

Figure 5. Proportion of behaviors (mean ± SE; $n = 3$) exhibited by *Crassostrea virginica* larvae at different ages (indicated by shading). Behavior types along the *x*-axis are wide helix (WH), helix (H), narrow helix (NH), straight (S), and diving (D), separated by behavior directions represented by the panels.

Salinity experiment

Quantitative metrics of behavior types did not differ between the salinity treatments (Table S1). This is contrary to previous studies, which found decreased swimming activity with decreasing salinity (Haskin, 1964). Those experiments induced gradual changes in salinity (replicating a tidal cycle of 6 h) or *in situ* measurements of the vertical position of larvae throughout tidal cycles. Another study using three clam species found that swimming speed varied with salinity, but this effect was not uniform across species and used static salinities with layered discontinuities rather than decreasing salinity over time (Mann *et al.*, 1991). Larval swimming in this study was measured in constant conditions after allowing for acclimation. Allowing larvae to acclimate may have removed the acute responses one would see, especially since acclimation time exceeds a tidal cycle in some treatments. Additionally, other studies (Haskin, 1964; Hidu and Haskin, 1978) categorized and quantified behaviors more broadly, grouping all upward or downward behaviors together into one average behavior, or simply accounted for active or inactive swimmers. The inconsistencies in methodology make it difficult to compare results of multiple studies. We recommend a standardization in how treatments are implemented and how swimming is measured and characterized in order to improve the ability to develop a more comprehensive model of bivalve larval swimming across species.

The relative frequency of behaviors observed varied between salinity treatments. Diving was observed most frequently in the lowest-salinity treatment (6 ppt) and may represent a mechanism to escape quickly to deeper, higher-salinity waters in salt-wedge estuaries. Field observations have found fewer larvae in the surface waters on ebb tides (low salinity) than on flood tides (high salinity) (Haskin, 1964). In the lowest salinity, fewer wide helix behaviors were observed. This may be due to larvae attempting to move away from low-salinity environments rather than searching for a better environment. Straight upward behaviors were observed more frequently in moderately high salinity (16 ppt), possibly as a mechanism for retention in a habitat with suitable salinity.

Ontogeny experiment

Larval swimming behavior changed as larvae aged, both in the frequency of observed behaviors and in the nature of those behaviors. For example, mean velocity and acceleration of diving increased with larval age. This is likely a result of larvae becoming stronger swimmers with age due to their larger size (Hidu and Haskin, 1978; Mann *et al.*, 1991). Helix width increased with age, possibly because, as larvae aged, they were increasing opportunities to search for settlement habitat or food resources while limiting changes to their vertical position (Maciejewski *et al.*, 2019) while the younger larvae searched less. Maintaining a wider helix may require greater energy costs or different morphology of which smaller, younger larvae might not be capable.

The relative frequency of downward swimming behaviors increased with age. Since older larvae are larger and heavier (Mann *et al.*, 1991), upward swimming could become more costly with age. Older larvae also may seek deeper habitats as they search for suitable settling habitat (Finelli and Wethey, 2003; Fuchs *et al.*, 2013, 2015), or, as the acclimation results in our study suggest, older larvae may be more tolerant of environmental variability and thus have less need to seek new habitat. An important caveat related to our experimental design is that our observations were made at a fixed location, in the middle of the experimental aquaria. Given that our data show increased downward swimming as larvae age, our results could be biased because older larvae that may spend more time nearer the bottom may not have been observed.

For the oldest larvae used in this study, downward behaviors were exhibited about three times as frequently as the upward behavior, with the reverse in the youngest larvae. This increase in downward behaviors should ultimately put larvae in contact with benthic settlement habitat (Fuchs *et al.*, 2013). However, our observations were different than an earlier study, which found that large (~320-μm) veliger larvae have been reported to spend about three times as much time swimming upward as downward (Gallager, 1993). Since our study was designed to measure the relative frequency of behaviors across treatments, tracking changes in individual larvae over time was not possible.

Surprisingly, we found diving behavior to occur in all age groups, despite diving being previously reported only in competent larvae (Finelli and Wethey, 2003; Fuchs *et al.*, 2013, 2015). Further, short spurts of diving (~1 mm) were observed interspersed with helical behaviors. These short dives did not appear to have a trigger and were too brief to meet our categorization criteria but are confirmed by Hidu and Haskin (1978), who also were unable to identify a cause. Our results suggest that diving may be related to more than settlement (Finelli and Wethey, 2003) since it was observed across all stages of larval development. Instead, it may serve as a predator avoidance mechanism (although predators were not included in these experiments), as a response to mechanical stimulation *via* particle contact (although all observations were made in filtered seawater), or a means to invoke tidal stream transport.

Larval dispersal

It has been previously believed that larval behavior was of lesser importance to overall larval dispersal than physical processes (Thorson, 1950; Korringa, 1952; Verwey, 1966), but today the importance of bivalve larval behavior is not in question, and dispersal models explicitly incorporate some elements of behavior (Dekshenieks *et al.*, 1996; North *et al.*, 2008; Narváez *et al.*, 2012). Some oyster dispersal models have used behavior parameters derived from other species entirely, like fish in Hubbard and Reidenback (2015), due to a lack of available data. Our results suggest that it may

be important to incorporate larval swimming behavioral responses related to salinity and larval age in dispersal models, even if simplified. The frequency, direction, velocity, and triggers of behaviors likely influence dispersal patterns that determine likelihood of larvae reaching suitable habitats (Haskin, 1964).

Conclusion

In this study, *C. virginica* larval swimming behaviors were categorized using a comprehensive key, which provided a more detailed characterization of behaviors than previous studies. Acclimation time and the frequency of these behaviors differed with salinity and larval age. Acclimation can play a role in dispersal if time swimming in low salinities or early ages is reduced and those larvae spend less time traveling in the water column. Behavior experiments that change larval environments on short timescales should allow animals sufficient time to acclimate before observations can be made. While the directionality of various larval swimming behaviors did not vary between salinities, the characteristics (*e.g.*, velocity and acceleration) and the frequency at which various behaviors were observed changed with larval age. Incorporating time to acclimation, changes in frequency of behaviors due to salinity and larval age, and more highly specified swimming behavior characteristics into larval dispersal models will give a better understanding of dispersal dynamics in estuaries and will be useful in predicting population dynamics in a changing climate.

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Literature Cited

Caracappa, J. C., and D. M. Munroe. 2019. Variability in swimming behavior among broods of blue crab (*Callinectes sapidus*) zoeae. *J. Exp. Mar. Biol. Ecol.* **518:** 151176.

Charmant, J. 2019. Kinovea. [Online]. Available: http://www.kinovea.org [2019, September 13].

Dekshenieks, M. M., E. E. Hofmann, J. M. Klinck, and E. N. Powell. 1996. Modeling the vertical distribution of oyster larvae in response to environmental conditions. *Mar. Ecol. Prog. Ser.* **136:** 97–110.

Delaware Bay OFS Salinity Nowcast. 2021. NOAA Tides and Currents. [Online]. Available: https://tidesandcurrents.noaa.gov/ofs/ofs_mapplots.html?ofsregion=db&subdomain=0&model_type=salinity_nowcast [2021, September 20].

Dove, M. C., and W. A. O'Connor. 2007. Salinity and temperature tolerance of Sydney rock oysters *Saccostrea glomerata* during early ontogeny. *J. Shellfish Res.* **26:** 939–947.

Eierman, L. E., and M. P. Hare. 2013. Survival of oyster larvae in different salinities depends on source population within an estuary. *J. Exp. Mar. Biol. Ecol.* **449:** 61–68.

Erftemeijer, P. L. A., J. K. L. van Beek, L. J. Bolle, M. Dickey-Collas, and H. F. J. Los. 2009. Variability in transport of fish eggs and larvae: modelling the effects of coastal reclamation. *Mar. Ecol. Prog. Ser.* **390:** 167–181.

Finelli, C. M., and D. S. Wethey. 2003. Behavior of oyster (*Crassostrea virginica*) larvae in flume boundary layer flows. *Mar. Biol.* **143:** 703–711.

Freire, C. A., and F. D. F. Sampaio. 2021. Stress and disease in aquaculture, and their effects on homeostasis and osmoregulation: the metabolic connection. Pp. 273–295 in *Aquaculture Pharmacology*, F. Kibenge, B. Baldisserotto, and R. Chong, eds. Elsevier Academic, London.

Fuchs, H. L., E. J. Hunter, E. L. Schmitt, and R. A. Guazzo. 2013. Active downward propulsion by oyster larvae in turbulence. *J. Exp. Biol.* **216:** 1458–1469.

Fuchs, H. L., G. P. Gregory, E. J. Hunter, A. J. Christman, and F. J. Diez. 2015. Hydrodynamic sensing and behavior by oyster larvae in turbulence and waves. *J. Exp. Biol.* **218:** 1419–1432.

Fuchs, H. L., J. A. Specht, D. K. Adams, and A. J. Christman. 2017. Turbulence induces metabolically costly behaviors and inhibits food capture in oyster larvae, causing net energy loss. *J. Exp. Biol.* **220:** 3419–3431.

Gallager, S. M. 1993. Hydrodynamic disturbances produced by small zooplankton: case study for the veliger larva of a bivalve mollusc. *J. Plankton Res.* **15:** 1277–1296.

Galtsoff, P. S. 1964. The American oyster *Crassostrea virginica* Gmelin. Fishery Bulletin 64. Fish and Wildlife Service, US Department of the Interior, Washington, DC.

Guo, X., Y. Wang, G. Debrosse, D. Bushek, and S. E. Ford. 2008. Building a superior oyster for aquaculture. *Jersey Shorel.* **25:** 7–9.

Guo, X., C. Li, H. Wang, and Z. Xu. 2018. Diversity and evolution of living oysters. *J. Shellfish Res.* **37:** 755–771.

Haskin, H. H. 1964. The distribution of oyster larvae. *Proc. Symp. Exp. Mar. Ecol.* **2:** 76–80.

Helm, M. M., and N. Bourne. 2004. *Hatchery Culture of Bivalves: A Practical Manual*. Food and Agriculture Organization of the United Nations, Rome.

Hidu, H., and H. H. Haskin. 1978. Swimming speeds of oyster larvae *Crassostrea virginica* in different salinities and temperatures. *Estuaries* **1:** 252–255.

Hubbard, A. B., and M. A. Reidenback. 2015. Effects of larval swimming behavior on the dispersal and

settlement of the eastern oyster *Crassostrea virginica*. *Mar. Ecol. Prog. Ser.* **535:** 161–176.

Korringa, P. 1952. Recent advances in oyster biology. *Q. Rev. Biol.* **27:** 539–565.

Maciejewski, M. F., K. S. Meyer, J. D. Wheeler, E. J. Anderson, N. C. Pittoors, and L. S. Mullineaux. 2019. Helical swimming as an exploratory behavior in competent larvae of the eastern oyster (*Crassostrea virginica*). *J. Exp. Mar. Biol. Ecol.* **510:** 86–94.

Mann, R., and J. S. Rainer. 1990. Effect of decreasing oxygen tension on swimming rate of *Crassostrea virginica* (Gmelin, 1791) larvae. *J. Shellfish Res.* **9:** 323–327.

Mann, R., B. M. Campos, and M. W. Luckenback. 1991. Swimming rate and responses of larvae of three mactrid bivalves to salinity discontinuities. *Mar. Ecol. Prog. Ser.* **68:** 257–269.

Maxwell, J. T., J. C. Bregy, S. M. Robeson, P. A. Knapp, P. T. Soulé, and V. Trouet. 2021. Recent increases in tropical cyclone precipitation extremes of the US east coast. *Proc. Natl. Acad. Sci. USA* **118:** e2105636118.

Munroe, D., A. Tabatabai, I. Burt, D. Bushek, E. N. Powell, and J. Wilkin. 2013. Oyster mortality in Delaware Bay: impacts and recovery from hurricane Irene and tropical storm Lee. *Estuar. Coast. Shelf Sci.* **135:** 209–219.

Najjar, R. G., H. A. Walker, P. J. Anderson, E. J. Barron, R. J. Bord, J. R. Gibson, V. S. Kennedy, C. G. Knight, J. P. Megonigal, R. E. O'Connor *et al.* 2000. The potential impacts of climate change on the mid-Atlantic coastal region. *Clim. Res.* **14:** 219–233.

Najjar, R., L. Patterson, and S. Graham. 2009. Climate simulations of major estuarine watersheds in the mid-Atlantic region of the US. *Clim. Change* **95:** 139–168.

Narváez, D. A., J. M. Klinck, E. N. Powell, E. E. Hofmann, J. Wilkin, and D. B. Haidvogel. 2012. Circulation and behavior controls on dispersal of eastern oyster (*Crassostrea virginica*) larvae in Delaware Bay. *J. Mar. Res.* **70:** 411–440.

North, E. W., Z. Schlag, R. R. Hood, M. Li, L. Zhong, T. Gross, and V. S. Kennedy. 2008. Vertical swimming behavior influences the dispersal of simulated oyster larvae in a coupled particle-tracking and hydrodynamic model of Chesapeake Bay. *Mar. Ecol. Prog. Ser.* **359:** 99–115.

R Core Team. 2021. R: a language and environment for statistical computing. [Online]. R Foundation for Statistical Computing, Vienna. Available: https://www.R-project.org [2021, October 25].

Sanderson, B. M., C. Wobus, D. Mills, C. Zarakas, A. Crimmins, M. C. Sarofim, and C. Weaver. 2019. Informing future risks of record-level rainfall in the United States. *Geophys. Res. Lett.* **46:** 3963–3972.

Shumway, S. E. 1996. Natural environmental factors. Pp. 467–513 in *The Eastern Oyster* Crassostrea virginica, V. S. Kennedy, R. I. E. Newell, and A. F. Eble, eds. Sea Grant College, College Park, MD.

Thorson, G. 1950. Reproductive and larval ecology of marine bottom invertebrates. *Biol. Rev.* **25:** 1–45.

Troost, K., R. Veldhuizen, E. J. Stamhuis, and E. J. Wolff. 2008. Can bivalve veligers escape feeding currents of adult bivalves? *J. Exp. Mar. Biol. Ecol.* **358:** 185–196.

Verwey, J. 1966. The role of some external factors in the vertical migration of marine animals. *Neth. J. Sea Res.* **3:** 245–266.

Wheeler, J. D., K. R. Helfrich, E. J. Anderson, and L. S. Mullineaux. 2015. Isolating the hydrodynamic triggers of the dive response in eastern oyster larvae. *Limnol. Oceanogr.* **60:** 1332–1343.

Wheeler, J. D., E. Luo, K. R. Helfrich, E. J. Anderson, V. R. Starczak, and L. S. Mullineaux. 2017. Light stimulates swimming behavior of larval eastern oysters (*Crassostrea virginica*) in turbulent flow. *Mar. Ecol. Prog. Ser.* **571:** 109–120.