Vol. 613: 97–106, 2019
https://doi.org/10.3354/meps12905

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published March 21

Low-salinity tolerance of early-stage oyster larvae from a mesohaline estuary

Robert J. Scharping[1,2,*], Louis V. Plough[1], Donald W. Meritt[1], Elizabeth W. North[1]

[1]University of Maryland Center for Environmental Science, Horn Point Laboratory, Cambridge, MD 21613, USA

[2]*Present address:* Department of Cell Biology, Microbiology, and Molecular Biology, University of South Florida, Tampa, FL 33620, USA

ABSTRACT: The eastern oyster *Crassostrea virginica* is an important ecosystem engineer which promotes biodiversity, yet some key physiological traits, such as the salinity tolerance of larvae in mesohaline regions, are not well understood. The objective of this study was to determine the salinity tolerances of early-stage *C. virginica* larvae of broodstock from the mesohaline Choptank River, Chesapeake Bay (USA), and to compare results with previous studies conducted with broodstock from the polyhaline Long Island Sound. Three experiments were conducted with broodstock and water from the Choptank River. After spawning, larvae were reared at salinities ranging from 3 to 26 for ~48 h post-fertilization. Salinity had a significant effect on larval survival in all experiments. While mean survival differed across experiments, the highest survival occurred between salinities of 7 and 16 in all experiments. The range of salinities which promoted high survival in this study was shifted lower by at least 7 salinity units compared to the range of salinities which promoted high survival of *C. virginica* larvae from the polyhaline Long Island Sound as reported in the literature. These results show that early-stage *C. virginica* larvae can survive at lower salinities than previously reported, and support the idea that the salinity of gametogenesis and the genetic background of broodstock influence the survival of larvae. In addition, this work provides the first quantitative estimates of absolute (instead of relative) survival of early-stage *C. virginica* larvae across salinities, which could be used to improve numerical models that support oyster management.

KEY WORDS: *Crassostrea virginica* · Physiological tolerance · Coastal resource management · Chesapeake Bay

1. INTRODUCTION

Across its extensive range, the eastern oyster *Crassostrea virginica* (Gmelin 1791) provides ecologically important services through water filtration and the creation of reefs (Newell 1988, Hargis & Haven 1999, Grabowski et al. 2012), and supports valuable commercial fisheries (Rothschild et al. 1994). Over the past century, populations have declined substantially due to overharvest, habitat loss, and disease (Haskin et al. 1966, Farley 1975, Kennedy & Breisch 1981, Ewart & Ford 1993, Rothschild et al. 1994, Wilberg et al. 2011). To revive the economic and ecological services that *C. virginica* reefs provide, modern restoration efforts are being implemented in polyhaline (salinities of 18–30) and mesohaline (salinities of 5–18) regions (Breitburg et al. 2000, Grabowski & Peterson 2007, Kennedy et al. 2011). Yet, basic information about the salinities which induce mortality of early-stage *C. virginica* larvae in mesohaline regions has not been established, which is important for understanding larval biology and ecology, and for modeling the dispersal and population dynamics of oysters across salinity regimes in US estuaries, including Galveston Bay (e.g. Klinck et al. 2002), Chesapeake Bay (e.g. North et al. 2010),

*Corresponding author: scharping@mail.usf.edu

Apalachicola Bay (e.g. Wang et al. 2008), Delaware Bay (e.g. Narváez et al. 2012), and Pamlico Sound (e.g. Puckett et al. 2014). The objective of this research was to fill this knowledge gap by conducting experiments with early-stage *C. virginica* larvae from the mesohaline Choptank River, Maryland, and to thereby advance understanding of environmental factors that shape the response of *C. virginica* to its environment.

Salinity is one of the most important factors affecting the distribution, productivity, and reproduction of *C. virginica* populations (Butler 1949, Shumway 1996). Adults and juveniles have a wide range of tolerance to salinity, with oysters typically found in salinities of 5–40 (Galtsoff 1964). Salinities where oysters are found in highest abundances usually range from ~14–28 and may vary geographically (Butler 1949, Chanley 1958, Galtsoff 1964, Shumway 1996). Salinity also exerts strong control over reproductive processes, such as gametogenesis and spawning of adult oysters (Loosanoff 1952, Shumway 1996). For example, *C. virginica* adults held in salinities of ~27 ceased gametogenesis when moved to salinities <5 at the onset of gonadal enlargement, but exhibited normal gametogenesis when placed back in slightly higher salinities between 7.5 and 12 (Loosanoff 1952).

Survival of *C. virginica* larvae is also strongly influenced by salinity, but reported salinity tolerances range widely among published studies, and most reports are observational rather than experimental. In the field, *C. virginica* larvae have been observed at salinities as low as 3 and up to 33 (e.g. Loosanoff & Engle 1938, Butler 1949, Carriker 1951, Davis 1958, Shumway 1996), although most of these studies did not focus on or observe the earliest larval stages. Only a few studies have focused on salinity tolerances of early-stage larvae (<48 h old) in lab-based experiments, and most of these were conducted with larvae produced from oysters conditioned at polyhaline salinities (Amemiya 1926, Davis 1958, Davis & Calabrese 1964, Eierman & Hare 2013). Amemiya (1926) examined salinity tolerance of *C. virginica* larvae produced from adults residing in the Alde-Ore Estuary near Orford, UK, (conditioned between 25 and 30) in laboratory experiments, but only exposed larvae to salinity treatments higher than 12. Davis & Calabrese (1964) examined the survival of *C. virginica* larvae from Long Island Sound adults (conditioned at 27) in salinity treatments ranging from 0–30, finding that larvae showed good survival close to their parental salinity (20–30) but suffered 100% mortality at salinities ≤12.5 at 27.5°C. In a more recent lab-based study, Eierman & Hare (2013) performed experiments of *C. virginica* larval salinity tolerance using oysters from Delaware Bay that were conditioned at 2 salinities (10 or 30) and reared larvae at the same 2 salinities.

Davis (1958) conducted the sole experimental study that examined the tolerance of early-stage *C. virginica* larvae to a wide range of salinities (2.5–27) using adults collected from mesohaline salinities (conditioned at 8.7 in upper Chesapeake Bay). Two day old larvae survived at salinities as low as 7.5, but for each treatment, relative survival (i.e. survival relative to the highest-surviving treatment) was reported rather than absolute survival (the proportion of larvae surviving compared to the initial number of fertilized gametes). Results of Davis (1958) suggest that *C. virginica* larval survival differs between mesohaline and polyhaline conditions, and that estimates of absolute survival in mesohaline regions are needed to improve quantitative understanding of the influence of environmental conditions on oyster populations.

If larval tolerances to mesohaline salinities vary among *C. virginica* populations, then the salinity tolerances previously reported may not reflect tolerances of oysters in other locations, such as the Choptank River, a mesohaline tributary of Chesapeake Bay. Cohorts of oyster larvae from Choptank River broodstock have high survival rates in the Horn Point Oyster Hatchery (HPOH) at salinities as low as ~9.5 (D. Meritt unpubl. data), well below the salinity (12.5) which induced 100% mortality of larvae from Long Island Sound broodstock (Davis & Calabrese 1964). This observation and the findings of Davis (1958) indicate that there is an important gap in knowledge of *C. virginica* larval salinity tolerances in mesohaline regions which, if filled systematically, would provide information needed to derive quantitative relationships between salinity and larval survival (e.g. Lough 1975). Thus, the objective of this study was to determine the survival of early-stage *C. virginica* larvae from the Choptank River at different salinities, and to compare results with those of experiments conducted with broodstock from the polyhaline Long Island Sound (Davis & Calabrese 1964) to examine differences in salinity tolerance between populations. To do so, the experimental design of Davis & Calabrese (1964) was followed using *C. virginica* broodstock from the mesohaline Choptank River to determine the upper and lower salinity tolerances for early-stage larvae from this population. Results have application for understanding the biology of *C. virginica* in mesohaline regions across the species' range, and could be used to support management tools such as oyster population and

habitat suitability models in mesohaline regions (e.g. Barnes et al. 2007, Narváez et al. 2012, Theuerkauf & Lipcius 2016, Puckett et al. 2018). In particular, differences in the upper and lower thresholds for larval survival could have major effects on mortality, connectivity, and suitability estimates over space and time.

2. MATERIALS AND METHODS

Three separate larval tolerance experiments were conducted, each with 8 treatment salinities which ranged from 3–26, following the experimental design of Davis & Calabrese (1964). In each experiment, fertilized eggs were derived from mass spawns of the adult broodstock collected from the Choptank River and placed in triplicate rearing chambers for each treatment salinity which were created with water collected from the Choptank River and Crystal Seas salt. The numbers of surviving and dead/deformed early-stage larvae were counted at the end of 2 d. Results were expressed as absolute survival, tested for significant differences between treatments, and converted to relative survival for direct comparison to Davis & Calabrese (1964).

2.1. Broodstock conditioning and spawning

Adult broodstock oysters were collected by HPOH staff from various reefs within the Choptank River (Fig. 1) and conditioned with flow-through water at salinities ranging from 10.0–11.2 over the spring/summer until spawning (Table 1). Spawns were performed with different groups of ripe adults (n = 50–100) that were placed in spawning tables and induced to spawn en masse by raising the water temperature, following standard hatchery techniques of the HPOH. Eggs and sperm were collected in separate buckets by identifying and removing females and males to their respective buckets when they were first observed to spawn on the spawning table. Adults finished spawning in their respective buckets and continued to be identified and retrieved until enough parents were present or until no additional spawning adults were found (~1 h). Gametes were then mixed for fertilization in 4 l buckets using an appropriate concentration of sperm and all eggs at the ambient salinity (10–12, similar to the conditioning salinities). Note that although every effort was made to remove spawning males and females as quickly as possible to their respective buckets to complete spawning, it is possible or likely that some females had ingested sperm before being removed to the female gamete bucket and thus some eggs were likely fertilized before the directed fertilization occurred. Nevertheless, these spawns produced a random mix of embryos with mixed parentage from multiple males and females (10s of parents). To determine the number of fertilized gametes from each spawn, 1 ml aliquots (n = 3) of gametes were counted

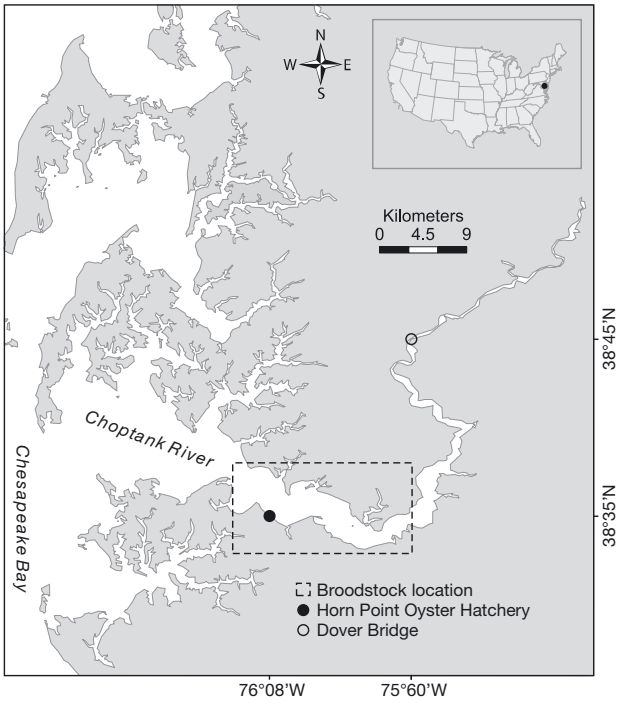

Fig. 1. Choptank River, a tributary of Chesapeake Bay in Maryland, USA. The locations of water collection sites at the Horn Point Oyster Hatchery and Dover Bridge are indicated, as is the area where adult broodstock was collected (dashed box). The map was downloaded from the IAN Image Library (ian.umces.edu/imagelibrary/), and was created by Tracey Saxby and Kate Boicourt. The inset of the continental US shows the location of Chesapeake Bay

Table 1. Experimental conditions summary, including the spawn date of each experiment, the location at which adult broodstocks were collected, Choptank River ambient salinity at which adults were spawned, and the mean ± SD experimental temperatures of the larvae-rearing vessels measured at the conclusion of each 48 h experiment

Experiment	Spawn date (2015)	Broodstock source	Salinity	Temperature (°C)
A	9 June	Black Buoy	11.2	25.7 ± 0.26
B	14 July	Black Buoy	10.0	26.2 ± 0.23
C	23 July	Black Buoy or Chloras Point	10.2	26.0 ± 0.36

from a known volume. The mean of the aliquots was used to calculate the concentration of gametes, which then was used to set the initial concentrations of larvae in each experiment.

2.2. Experiments

Three separate larval tolerance experiments (hereafter referred to as Experiments A, B, and C) were conducted in a temperature-controlled chamber that maintained water temperatures of ~26°C (Table 1), simulating the average summer water temperatures in the Choptank River (Goodwin 2015). Within 2–4 h of fertilization, ~30 000 mass-spawned embryos were transferred directly from their salinity of origin into 3 l glass rearing vessels (n = 24), with initial concentrations of larvae set at 10 000 ind. l^{-1} (similar to the experiments of Davis & Calabrese 1964). Treatments were set at salinities of 3, 5, 7, 9, 11, 16, 21, and 26. To simulate conditions experienced by larvae in the Choptank River, the lower-salinity treatments (3, 5, 7, 9) were created by adding Crystal Seas brand artificial sea salt to water that was collected from the upper Choptank River near the Dover Bridge (highway MD-331; Fig. 1), which ranged in salinity from 1.0–2.9. The higher-salinity treatments (11, 16, 21, 26) were created by starting with water that was collected from HPOH (Fig. 1), which ranged in salinity from 9.8–11.0, and adding Crystal Seas salt to achieve the desired salinity. Treatment salinities were verified with a YSI 85 conductivity probe. Water was collected 2–7 d prior to each experiment, filtered through a 1 µm filter, refrigerated in the dark, and filtered again through a 1 µm filter before use. Each treatment in each experiment was run in triplicate (technical replicates to account for potential tank effects or other variation in experimental execution) for a total of 24 vessels (3 l each) in each experiment, and 72 vessels over all experiments. Rearing vessels were aerated with a steady stream of bubbles from air pumps to gently perturb the water surface. Expt A was conducted under fluorescent lights on a 12 h light, 12 h dark cycle, whereas Expts B and C were run in the dark.

Similar to Davis & Calabrese (1964), the experiments were ended after 48 h, well past the time needed for eggs of *Crassostrea virginica* to develop into straight-hinged (prodissoconch I) larvae (Kennedy et al. 1996), and the larvae were not fed. Larvae from each vessel were filtered on a 44 µm sieve and were preserved using 95% buffered ethanol (pH = 8.0–8.5). Reported survival explicitly reflects the number of larvae retained on the 44 µm sieve at 48 h. Preserved larvae were rinsed into a specified volume of deionized water before they were counted using a light microscope. Larvae were counted over a span of 6–28 d, 6–15 d, and 5–9 d post-preservation for Expts A, B, and C, respectively.

Preserved larvae were counted from 1 ml aliquots (n = 4) taken from each well-mixed sample and were characterized based on the shell symmetry, shape, and the rough amount of tissue they retained. If the proportion of surviving larvae in a sample was below ~6% of initial concentrations, or if the coefficient of variation among the aliquot counts was very high (>0.1), a census of the whole sample was conducted. Larvae were counted as alive if their shell was filled with pigmented tissue. Larvae were counted as dead if their shells were empty, contained large air bubbles, or contained severely degraded tissue. In the rare instance that larvae had partially filled shells with intact tissue (larval tissue degrades rapidly once larvae die), they were counted as alive if larval tissue mostly filled the shell area and looked intact. Some shrinkage of tissue into the shell can occur with ethanol preservation, but larvae were examined within 4 wk post-preservation so that determination of live/dead larvae was clear.

2.3. Analysis

The proportion of larvae that survived in each replicate and experiment was plotted using Microsoft Excel, and statistics were conducted with R v.3.1.2 (R Development Core Team 2008). Proportion survival was calculated by dividing the number of live larvae by the initial number of embryos in each vessel. ANOVA and Tukey's honestly significant difference statistical tests were conducted to determine if the proportion of surviving larvae differed between treatments. Calculated proportions of surviving larvae from each replicate were arcsine square root transformed before being used in statistical tests. Transformed data passed Shapiro's test for normality and Levene's tests for homogeneity of variance. The fraction of dead and deformed larvae present at the end of each experiment also was calculated. To do so, the number of larvae considered dead and deformed at the end of each experiment was summed and then divided by the total number of larvae present at the end of each experiment.

The relative percentage of surviving larvae was calculated to enable direct comparison with results from the experiments of Davis & Calabrese (1964)

conducted at 27.5°C. Relative percent survival was calculated as the percentage of the number of surviving larvae in a given treatment, relative to the survival of the treatment with the highest survival in each experiment, set at 1.0 or 100%.

3. RESULTS

Across the 3 independent salinity exposure experiments, overall larval survival differed substantially, with Expt A showing the highest survival (up to 68% absolute survival at a salinity of 9), while the highest survival in a treatment for Expts B and C was 33 and 27%, respectively, both at a salinity of 11 (Table 2, Fig. 2). Larval survival varied significantly across salinity treatments, with an average of 0% survival at a salinity of 3, 1% survival (±1% SD) at 5, 32–40% survival (±16–24%) between 7 and 16, 17% survival (±19%) at 21, and 3% survival (±5%) at 26 (Table 2, Fig. 2). One-way ANOVA indicated that salinity had a significant effect on larval survival in each experiment ($p < 0.001$, and $F = 20.74$, 59.56, and 104.4 for Expts A, B, and C, respectively). Within experiments, average survival of the mid-range salinity treatments (salinities of 9–16) was typically significantly different from the lower (<7) and higher (>21) salinities (significant Tukey's pairwise comparisons; see Fig. 2). This indicated an optimal survival range between 7 and 16. More specifically, the highest percent (absolute) survival within these optimal ranges was 68, 33, and 27%, for Expts A, B, and C, respectively. The highest fraction of dead and deformed larvae, many of which had reached the straight-hinge stage, were found in salinity treatments of 3, 5, and 26 (Table 2), which also had the lowest survival (Table 2).

Table 2. Mean ± SD proportion of surviving larvae and mean fraction of larvae that were dead or deformed at the end of the 2 d experiments (Expts A, B, and C). Mean fraction dead or deformed for each replicate was calculated out of the total number of larvae observed in each treatment at the end of the experiment. The grand mean ± SD for each salinity treatment was calculated with data pooled from all experiments

Salinity	A	B	C	Grand mean
Mean proportion of surviving larvae				
3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
5	0.03 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01
7	0.58 ± 0.03	0.29 ± 0.05	0.08 ± 0.03	0.32 ± 0.22
9	0.68 ± 0.06	0.17 ± 0.04	0.24 ± 0.02	0.36 ± 0.25
11	0.60 ± 0.02	0.33 ± 0.01	0.27 ± 0.04	0.40 ± 0.16
16	0.66 ± 0.05	0.17 ± 0.02	0.20 ± 0.03	0.34 ± 0.24
21	0.33 ± 0.23	0.13 ± 0.05	0.05 ± 0.01	0.17 ± 0.19
26	0.10 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.05
Mean fraction of dead or deformed larvae				
3	0.67 ± 0.58	0.00 ± 0.00	0.30 ± 0.27	0.32 ± 0.43
5	0.12 ± 0.02	0.25 ± 0.22	0.16 ± 0.11	0.18 ± 0.14
7	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.02	0.03 ± 0.02
9	0.01 ± 0.00	0.14 ± 0.02	0.03 ± 0.01	0.04 ± 0.05
11	0.01 ± 0.00	0.09 ± 0.01	0.03 ± 0.02	0.04 ± 0.04
16	0.00 ± 0.00	0.09 ± 0.03	0.06 ± 0.01	0.05 ± 0.04
21	0.02 ± 0.01	0.14 ± 0.03	0.11 ± 0.01	0.09 ± 0.06
26	0.19 ± 0.06	0.25 ± 0.10	0.40 ± 0.37	0.23 ± 0.15

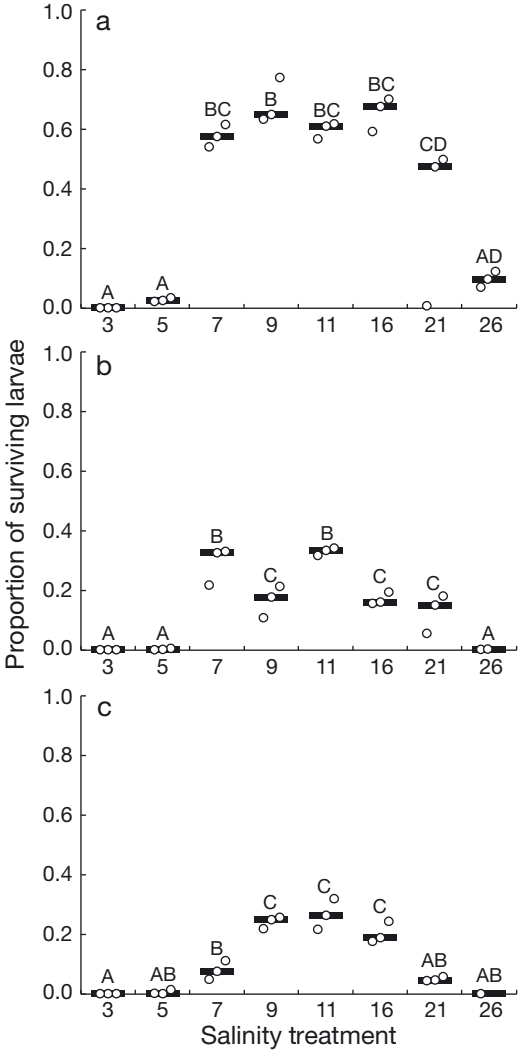

Fig. 2. Proportion of surviving *Crassostrea virginica* larvae from the Choptank River after 48 h over a range of salinity treatments during Expts (a) A (9–11 June 2015), (b) B (14–16 July 2015), and (c) C (23–25 July 2015). Circles indicate individual replicates from each treatment, and black bars represent the median of the replicates for each treatment. Similar letters above each treatment indicate that the treatment means were not significantly different based on Tukey's HSD test with arcsine square root transformed data. Data points within each salinity treatment are shifted slightly to prevent overlap

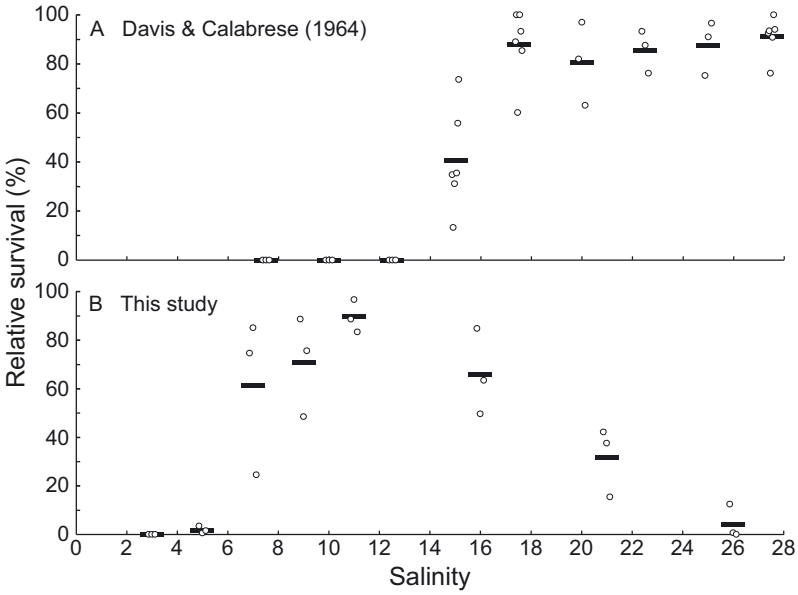

Fig. 3. Relative percentage of surviving *Crassostrea virginica* larvae after 48 h over a range of salinity treatments using larvae spawned from broodstock from (A) Long Island Sound (redrawn from Davis & Calabrese 1964, their Table 12, 27.5°C treatments) and (B) the Choptank River (this study, conducted at 26°C). Relative percentage was calculated by dividing the number of straight-hinged larvae in each treatment by the highest number of surviving straight-hinged larvae in the experiment, then multiplying by 100. Circles indicate the mean of replicates from individual experiments and black bars represent the mean from all experiments. Adults from Long Island Sound were conditioned at a salinity of ~27 (Davis & Calabrese 1964), whereas those from the Choptank River were conditioned at salinities between 10.0 and 11.2. Data points within each salinity treatment are shifted slightly to prevent overlap

Comparison of relative percent survival between these experiments and those of Davis & Calabrese (1964) indicated that the minimum salinity which promoted high survival in this study was at least 7 salinity units lower than the minimum salinity which promoted high survival of *C. virginica* larvae from the polyhaline Long Island Sound (Davis & Calabrese 1964) (Fig. 3). Also, the high salinity that induced 97 ± 5% mortality in this study (salinity of 26) did not produce significant mortality in Davis & Calabrese (1964) (salinities of 25 and 27.5) (Fig. 3).

4. DISCUSSION

While it is widely accepted that eastern oysters have a remarkable capacity to tolerate a range of salinities (e.g. Shumway 1996), surprisingly little experimental data exist on the tolerances of larvae from various salinity regimes, particularly for larvae from mesohaline environments that are typical of some of the most productive populations of the species (e.g. Chesapeake Bay). The primary findings of this study were (1) that oyster larvae survived at lower salinities than previously reported, and (2) that the range of highest survival of larvae from the mesohaline Choptank River occurred at substantially lower salinities than the range which resulted in highest survival of larvae from polyhaline Long Island Sound (Davis & Calabrese 1964), similar to results of Davis (1958). In our experiments, the highest larval survival occurred at salinities between 7 and 16, a range centered around the salinities at which their respective broodstock were conditioned (10.0–11.2). In contrast, Davis & Calabrese (1964) reported 100% mortality of larvae at salinities ≤12.5 and highest larval survival at salinities ~17–25 (close to the salinity at which their adult oysters were conditioned) (Fig. 3). Moreover, our finding of >90% mortality at a salinity of 26, a salinity at which Long Island Sound oyster larvae survived quite well (Davis & Calabrese 1964), suggests that Choptank River oysters were better acclimated to the local, low-salinity parental environment.

Our results support the observations of Davis (1958) and the more recent findings of Eierman & Hare (2013), who showed that oysters which undergo gametogenesis in mesohaline environments produce larvae that survive better in similar mesohaline conditions compared to larvae from oysters that undergo gametogenesis in more saline waters. The variation in larval salinity tolerances observed across oyster populations may be due to the acclimation of larvae to their parental conditioning environment (Davis 1958), but it also may have a genetic, adaptive basis. Results from Eierman & Hare (2016) suggest that populations of oysters from low-salinity regions might have different patterns of gene expression in response to salinity change than those from higher-salinity regions, but whether this is caused by adaptive differences or plastic, acclimatory responses is challenging to demonstrate unequivocally.

While larvae from Choptank River broodstock appear to tolerate much lower salinities compared with those from Long Island Sound oysters, it is not known whether absolute survival rates differed between studies because Davis & Calabrese (1964) reported results only as relative percent survival (all

values normalized to the highest surviving treatment, which is reported as 100%). The authors reported relative estimates of survival because they found substantial variation in survival between experiments, as was found in our study. Such variation in larval survival among cultures or experiments is not unexpected because of the many factors that can influence gametogenesis and larval survival, including adult condition, broodstock source, timing in the spawning season, and temporal/spatial differences in water chemistry (Thompson et al. 1996). We chose to report absolute survival to provide new and useful information for models of larvae in mesohaline regions.

It is possible, however, that aspects of our experimental design may have contributed to the high variation in survival observed among experiments. For example, the absence of light during larval rearing in Expts B and C may have had negative effects on larval survival that were not a factor in Expt A, which showed higher overall survival. It seems unlikely that a lack of light would have had serious negative effects on larval survival during this early period in which larvae are non-feeding, and it is more likely that the depressed survival was due to random cohort effects, water quality differences, or other environmental causes. In another marine bivalve species (*Mytilus edulis*), experiments in which larvae were exposed to darkness over the entire larval period (i.e. not just the first 48 h) did cause slightly lower survival (but not growth) compared with a 12:12 h light:dark cycle treatment (Nielsen & Strömgren 1985).

Using a 44 µm mesh to sieve larvae also may have produced a downward bias in our larval survival estimates, but this would not explain the major differences in survival at the optimal salinity range (7–16) among our experiments, because the same mesh was used for Expts A, B, and C. Nevertheless, the mesh size we used may have allowed very slow-developing larvae to be missed (and hence our survival measure explicitly reflects the number of live larvae retained on the 44 µm mesh). Notably, Davis & Calabrese (1964) and other workers did not report the mesh size they used, and there is some conflict in the literature about the appropriate mesh size for sampling straight-hinged *Crassostrea virginica* larvae (e.g. Stanley & Sellers 1986, Helm & Bourne 2004, Wallace et al. 2008). Future studies could include a screen with mesh size that is small enough to ensure capture of all slow-growing live embryos at 48 h post fertilization (e.g. 25 µm mesh). This small screen would allow testing for an effect of slow growth, and, when coupled with a screen of 44 µm mesh, would allow direct comparison with results from this study.

Variation in the quality or chemistry of water used in experiments could also have played a role in the differential survival across experiments. Experiments were conducted over a 6 wk period, and for each one, water was collected from the Choptank River before being filtered at 1 µm. Thus, significant changes in water quality due to recent algal blooms, rain events, or other major environmental events could have affected water quality in unknown ways, and would have been difficult to detect. The use of artificial seawater across experiments would likely have reduced the potential for variation in water chemistry over time, but artificial seawater diluted to estuarine-like salinities with deionized water or well water may produce water chemistry conditions that are not conducive to larval growth (e.g. due to low aragonite saturation states; Waldbusser et al. 2015) or are not representative of the natural estuarine environment. The use of local water makes results of the experiment more applicable to the local conditions.

Another important consideration in our experimental design was the relative role of acute osmotic shock to embryos when they were first introduced to the salinity treatments. Although following the protocol of Davis & Calabrese (1964) allowed direct comparison between experiments, some of the treatments may not represent a stress that larvae would experience in nature, and the rapid salinity change could have caused mortality even if the treatment salinity was not stressful (e.g. Shumway 1996). Even though some treatments may have represented more extreme rates of salinity change compared to what *C. virginica* larvae typically experience in the environment, there are instances in nature when the salinity in *C. virginica* habitats can change rapidly over small spatial scales on changing tides or during major rain and storm events. Tropical storm Agnes struck the Chesapeake Bay at the start of the 1972 oyster spawning season and caused rapid reductions in the salinities of lower-Bay tributaries that actually reversed tributary salinity gradients, and was detrimental to adult oyster survival, reproduction, and larval setting (Cory & Redding 1976, Haven et al. 1976). Although it is not possible to rule out the role of acute osmotic stress when simply moving gametes to a given salinity treatment in an experiment, the presence of dead straight-hinged larvae on Day 2 in our extreme-salinity treatments indicated that mortality was not immediate for all larvae — some developed to the straight-hinged stage (Table 2). Using gradual initial salinity shifts when transferring embryos from spawning salinities to treatment vessels could have resulted in less osmotic shock and higher

larval survival. Therefore, the estimates of survival reported here likely are conservative, especially for the lowest and highest treatments. Future studies of oyster larval salinity tolerance could use a more gradual salinity change so that potential stress due to osmotic shock can be minimized (e.g. Anger 1996, Khatooni et al. 2011).

Overall, the results of our experiments show that eastern oyster larvae can survive at very low salinities. This work significantly advances our understanding of the remarkable physiological capacity of eastern oyster larvae and, particularly, how oysters respond to rapid changes in salinity. However, we must caution that these results come from a series of laboratory studies with relatively limited experimental complexity (e.g. 1 broodstock source population, 1 conditioning regime, only early-stage larvae), under initial salinity exposures that may have changed more rapidly than what is typically seen in nature. It is important to note that older (veliger) larvae may have an even greater tolerance to low (or high) salinity (e.g. Priester 2016). Early-stage oyster embryos/larvae were chosen for this experiment because they are thought to be the most vulnerable. It is also possible that the range of salinity tolerance of early-stage oyster larvae from the Choptank River or other mesohaline populations could be even greater, with survival possibly below the salinity of 5 observed here, such as in Barnegat Bay, New Jersey, where early-stage larvae were collected in salinities as low as 3.1 (Carriker 1951). Recently spawned adults and newly settled juvenile oysters have been observed at sites in the Choptank River that routinely experience salinities around or below 5 (D. Meritt pers. obs.); thus, there may yet be un-sampled populations of oysters that can produce larvae with even more impressive tolerances to extreme low salinities. The importance of conditioning salinity (and other factors associated with gametogenesis) for larval performance also remains to be explored fully for mesohaline or low-salinity oysters. While previous studies report that gametogenesis ceases in oysters held at salinities of 5 or lower (Loosanoff 1952, Shumway 1996), most of these observations were not made on adults from low-salinity environments. More complex larval experiments that involve multiple population (broodstock) sources conditioned at multiple salinities would improve understanding of the relationships between local adaptation and acclimatization and gametogenesis timing, egg quality, and larval performance at various salinities. There is still much work to be done to understand the biology and physiological tolerances of oyster larvae, and we hope that this study will provide renewed interest in the experimental study of the complex early life history dynamics of *C. virginica* across environmental gradients.

In addition to enhancing knowledge of early-life dynamics of *C. virginica*, results of this study also provide valuable information for parameterizing numerical models that can be applied to better understand larval growth (e.g. Dekshenieks et al. 1993), larval transport (e.g. North et al. 2008, Narváez et al. 2012, Spires 2015), spatially resolved population dynamics (e.g. Klinck et al. 2002, Wang et al. 2008), and habitat suitability (e.g. Barnes et al. 2007, Theuerkauf & Lipcius 2016, Puckett et al. 2018) of *C. virginica* by providing survival rates in mesohaline regions. Modeling studies of oysters in a particular salinity range or habitat would benefit greatly from empirical data on salinity tolerance (or tolerance to other environmental factors) at multiple developmental stages generated from laboratory experiments using local broodstock. Empirical data on optimal salinity conditions for oyster larvae from a mesohaline environment also provide valuable information for maximizing performance and survival of larvae produced from hatcheries in these regions. While further investigation of the interaction between temperature and salinity is needed, as well as the relative influence of adult acclimation vs. adaptation in buffering larval responses to salinity change, this study sets the stage for future experimental work in this arena, and provides an important set of data and results for the salinity response of larvae from mesohaline regions. Overall, this study confirms the importance of considering local environmental factors when managing oyster populations, and provides quantitative estimates of both absolute and relative survival.

Acknowledgements. We thank Stephanie Alexander, Bob Carey, Anna Priester, and the crew of the Horn Point Oyster Hatchery for supplying fertilized embryos for these experiments. We also thank Jacob Goodwin and Jason Spires for their advice and guidance; Connor Reyer and Alison Aceves for help with water collection and sample preservation; and anonymous reviewers for their comments which strengthened this manuscript. This research was funded by the National Science Foundation (NSF) Division of Ocean Science and Maryland Sea Grant via the Research Experiences for Undergraduate Program (OCE-1262374), by an NSF grant to E.W.N. (OCE-1427019), and by support from the Deerbrook Charitable Trust (DCT 15-30) to L.V.P. This is UMCES contribution number 5539.

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Editorial responsibility: Romuald Lipcius, Gloucester Point, Virginia, USA

Submitted: July 16, 2018; Accepted: February 20, 2019
Proofs received from author(s): March 18, 2019