

Research Article

Growth of Oyster (*Crassostrea virginica*) Larvae in Small-Scale Systems Using an Algae Concentrate Food Source

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Replicated studies are advantageous for optimizing larval rearing of the Eastern oyster (*Crassostrea virginica*) and increasing the availability of high-quality seed for the continued expansion of the U.S. oyster aquaculture industry. Although small-scale systems using live algal feeds have been used successfully, rearing larvae on algae concentrate presents additional challenges. To determine the feasibility of rearing oyster larvae in small-scale systems using algae concentrate, oyster larvae were raised for 2 weeks in replicate control (1,000 L) and microcosm (17 L) tanks. Five aeration strategies were tested in the microcosms in two separate trials. Results of this study indicate similar survival in small systems compared to controls through the appearance of eyed larvae. Accumulated algae and pink biofilm formation in microcosms using polyvinyl chloride (PVC) airlifts suggest that this aeration strategy is undesirable. One- and 5-mL air injectors maintained higher overnight oxygen levels than controls. The recovery of more eyed larvae after 14 dpf in control systems may be the result of significant temperature fluctuations in microcosms. Overall, this study demonstrates that algae concentrate can be used to rear oyster larvae in small-scale systems, providing a live feed alternative that saves space and labor in replicated studies.

1. Introduction

Natural oyster populations have been in decline globally for decades. Estimates indicate that only 84% of historical oyster reefs are currently extant [1] due to a variety of factors including habitat degradation, eutrophication, and overfishing [2]. As natural populations have declined, oyster aquaculture has grown with molluscan aquaculture accounting for ~30% of all global marine aquaculture production by weight in 2021 [3]. In the United States, 181,517 metric tons of marine mollusks were produced in 2021 at a value of \$29.9 billion dollars [3].

Oyster aquaculture is well established on the Pacific and Atlantic coasts of the United States and is a relatively new and growing industry in the Gulf of Mexico (GOM). A common bottleneck for the GOM oyster aquaculture industry is reliable access to oyster seed. Large-scale oyster hatcheries are needed to support the industry but require large upfront costs and rely on proper site selection because water quality has a significant impact on larval rearing success [4, 5].

Developing methods for rearing oyster larvae on a small scale will allow for replicated scientific studies to optimize and increase hatchery production in the region.

Standard oyster hatchery methods use larval rearing tanks that are 250-L or larger, which can necessitate a large spatial footprint and limit the capacity for scientific replication [6–8]. Research demonstrates the potential to rear oyster larvae to settlement using live feeds in microcosms (15–20 L) [9–12], allowing for a comparison of multiple factors without the need for large rearing tanks. While small-scale shellfish larval rearing setups have been used successfully, little has been published on the efficiency of these methods in comparison to more typical, large-scale larval rearing systems. Furthermore, the previously mentioned setups require substantial labor and space costs associated with maintaining live feed cultures [13].

Oyster larvae rely on a steady and diverse supply of microalgae to feed on throughout the larval cycle [7, 14–16]. Monoculture of live microalgae is the predominant choice for oyster hatcheries and involves the culturing of several different

microalgae species to meet the nutritional and dietary needs of oyster larvae [7, 15, 17]. While this method is cost-effective for large hatcheries, it is a time-consuming and expensive process that requires specific expertise and can limit the ability of small-scale hatcheries to operate efficiently [15]. Commercially available microalgae concentrates, consisting of an assemblage of species targeted in monoculture, have been used successfully to rear oyster larvae and allow hatcheries to bypass full-scale microalgae production [16, 17]. Microalgae concentrates have similar costs to live cultures [13]; however, they have a small footprint, long shelf life, and do not require trained personnel for maintenance [18]. Despite the benefits of these concentrated feeds, there are potential concerns with their use in hatcheries [18]. As the concentrates are composed of dead algal cells, they do not supplement oxygen in larval tanks, a benefit provided by live feed. Without proper mixing, dead algal cells settle out of the water and stick to surfaces, providing excess nutrients that may trigger bacterial blooms. These concerns may be exacerbated in smaller systems, in part due to their larger surface area to volume ratio. To date, the effectiveness of concentrates in small-scale oyster hatchery systems has not been evaluated.

This study provides a direct comparison between rearing oyster larvae in microcosms versus in standard, large-scale systems using microalgae concentrate. Varying methods of aeration are assessed to determine optimal methods for rearing larvae on a small scale. Assessing the effectiveness of small-scale larval culture with microalgae concentrates is an important step in increasing replication for scientific research purposes, leading to optimized protocols for seed production to support the growing oyster aquaculture industry.

2. Materials and Methods

2.1. Large-Scale Systems. Large-scale control systems used in this study were 1,000 L blue plastic cone-bottom tanks (Poly-tank, Inc). Tanks were filled with ~1,000 L of natural seawater filtered to $1\ \mu\text{m}$. These were outfitted with a 2-inch standpipe to generate a static system (Figure 1). To create an airlift, a 4-inch polyvinyl chloride (PVC) pipe with a scalloped end was placed over the standpipe, scalloped end down. Two holes for the airline attachment were located ~5 cm from the bottom of the airlift. Aeration through the airline maintained water flow in the tank at a rate that produced water rings that dissipated before contacting the edge of the tank so as not to force larvae into the tank walls. Oyster larvae were batch fed twice daily with an algae concentrate (Shellfish Diet 1800[®], Reed Mariculture, Campbell, CA, USA). To prepare stock solutions of algae concentrate, ChlorAm-X (Reed Mariculture, Campbell, CA, USA) was dissolved in a pure sodium chloride solution (30 ppt) at 0.12 g ChlorAm-X per mL of algae concentrate. This product acts as a water conditioner and neutralizes ammonia, chlorine, and chloramines. The appropriate volume of algae concentrate was diluted in the ChlorAm-X/sodium chloride solution and then filtered through a $20\ \mu\text{m}$ bag filter to disperse clumped algal cells. The algae mixture was further rinsed through the bag filter with filtered ($1\ \mu\text{m}$) natural seawater. The larval tank system was stocked to reach a targeted cell

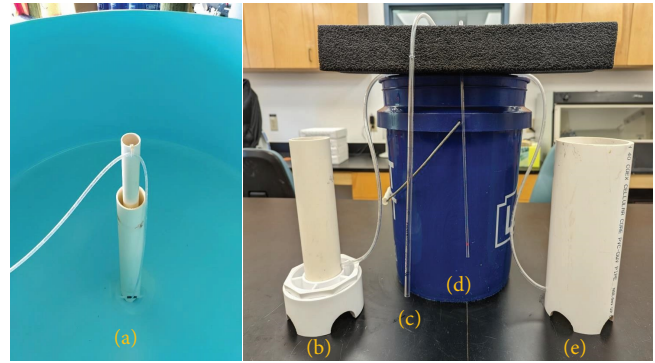


FIGURE 1: Aeration treatments used in this experiment. (a) Large-scale controls, (b) 2-inch airlift, (c) 5 mL air injector, (d) 1 mL air injector, and (e) 4-inch airlift.

density in the larval tanks of 20,000 cells mL^{-1} through 5 days post fertilization (dpf), 30,000 cells mL^{-1} through 9 dpf, and 40,000 cells mL^{-1} for the remainder of the trials.

2.2. Small-Scale Systems. Small-scale systems used in this study were buckets filled with 17 L of natural seawater filtered to $1\ \mu\text{m}$. Two trials occurred in late summer 2022 to test the impacts of different aeration types. In trial 1, the three aeration types tested were a 2-inch airlift, a 4-inch airlift, and a 1 mL air injector (Figure 1). The 2-inch airlift was constructed from a 2-inch PVC pipe inserted into a 4-inch \times 2-inch PVC reducer bushing base with a scalloped end, similar to large-scale systems. A hole for the airline was drilled directly above the bushing in the PVC pipe (~7 cm from the airlift base). The 4-inch airlift was constructed from a scalloped 4-inch PVC pipe, with the airline hole drilled ~3 cm from the base. For both airlifts, the airline was inserted until it reached the center of the pipe. The 1 mL air injector consisted of a 1 mL serological pipette with airline tubing attached to the tapered end. The pipette was stabilized centrally in the bucket by inserting it into a rectangular expanded polystyrene (EPS) foam support that rested on the top of the bucket. In trial 2, the three aeration types included the 1 mL air injector, a 5 mL air injector with the same structure as the 1 mL air injector but using a 5 mL serological pipette, and zero aeration. In all aerated treatments, the air was adjusted to allow for a steady stream of bubbles which generated similar water ring movement as the large-scale systems. Larvae were fed algae concentrate, processed as described above, in two batch feedings per day. The daily feed concentrations were the same as those in the large-scale systems.

2.3. Larval Rearing. Adult diploid Eastern oysters were spawned at the Auburn University Shellfish Lab (AUSL) in Dauphin Island, Alabama. Oysters were placed into individual tanks and spawning was stimulated via thermal manipulation and pheromone exposure [6]. Fertilized eggs were stocked into triplicate large-scale systems and triplicate small-scale systems of each aeration type at 10 larvae mL^{-1} . Filtered seawater changes occurred every other day until the first eyed larvae were seen, at which point water changes occurred daily. At each draining, the tanks, aerators, and airline tubing were cleaned with a mild bleach solution and

TABLE 1: Larval parameters measured in controls and microcosms throughout the 14-day trials.

Treatment	Larval growth rate ($\mu\text{m day}^{-1}$)	Days to first eyed larvae	Total eyed larvae (larvae mL^{-1})
Trial 1			
Control	$7.67 \pm 1.85^{\text{b}}$	13.0 ± 0.0	0.04 ± 0.02
2-inch airlift	$11.4 \pm 2.02^{\text{ab}}$	13.3 ± 1.2	0.07 ± 0.08
4-inch airlift	$10.4 \pm 1.14^{\text{a}}$	12.7 ± 0.6	0.14 ± 0.07
1 mL air injector	$12.0 \pm 1.22^{\text{a}}$	12.3 ± 0.6	0.38 ± 0.39
<i>p Value</i>	0.042	0.400	0.137
Trial 2			
Control	$14.0 \pm 2.26^{\text{a}}$	12.0 ± 0.0	$1.21 \pm 0.15^{\text{a}}$
1 mL air injector	$10.3 \pm 0.53^{\text{b}}$	13.0 ± 1.0	$0.22 \pm 0.19^{\text{b}}$
5 mL air injector	$10.4 \pm 0.60^{\text{b}}$	13.0 ± 0.0	$0.38 \pm 0.13^{\text{b}}$
Zero aeration	$10.1 \pm 0.59^{\text{b}}$	13.0 ± 0.0	$0.32 \pm 0.11^{\text{b}}$
<i>p Value</i>	0.014	0.095	<0.001

Bold *p Values* indicate significant differences among treatments ($p < 0.05$), with pairwise comparison results denoted using superscripts.

rinsed with freshwater and filtered seawater prior to refilling. To maintain appropriate stocking densities as the larvae grew, densities were reduced to 5 larvae mL^{-1} at 2 dpf and 4 larvae mL^{-1} at 6 dpf. At 8 dpf, larvae were size graded through a $75 \mu\text{m}$ sieve to remove slower growing individuals. Eyed larvae were removed using a $200 \mu\text{m}$ sieve.

2.4. Sampling. Trials continued for 14 dpf. Water quality (temperature and salinity) was measured in the AM ($\sim 09:00$) and PM ($\sim 17:00$) and after each water change ($\sim 12:00$). In trial 2, dissolved oxygen was also monitored at these time points. At each water change, larvae were counted volumetrically and shell length measurements (μm) of 10 larvae per tank were determined using a microscope with a calibrated reticle prior to restocking. When eyed larvae were observed, these were counted separately. The number of days to the first appearance of eyed larvae was recorded for each replicate. The total number of eyed larvae was standardized between large- and small-scale systems and reported as eyed larvae mL^{-1} .

2.5. Data Analysis. All data are reported as mean \pm SD. Shell lengths of technical replicates (i.e., individual larvae) within a replicate tank were averaged to obtain the size at each sampling day up to the appearance of the first eyed larvae. Growth rate was determined for each replicate tank in $\mu\text{m day}^{-1}$. As the first length measurements occurred at 2 dpf, this age is treated as the initial measurement.

$$\text{Growth rate } (\mu\text{m day}^{-1}) = \frac{\text{Length at } n \text{ dpf} - \text{length at 2 dpf}}{n - 2} \quad (1)$$

Larval size, growth rate, and survival (larvae mL^{-1}) at each sampling day, number of days to first eyed larvae, and total number of eyed larvae were compared across treatments using one-way analysis of variance (ANOVA). Overnight changes in temperature, salinity, and dissolved oxygen were calculated by subtracting the morning readings from the readings the evening prior. Morning, evening, post-water change, and overnight change in water quality parameters were also compared across treatments using one-way ANOVAs (Tukey's HSD post hoc).

Data were tested for homogeneity of variance and model residuals were tested for normality. In trial 1, days to first set was square root transformed to meet the assumption of normality. In trial 2, days to first eyed larvae could not meet normality with transformations, so a nonparametric Kruskal–Wallis test was used. All post-water change water quality parameters failed normality and were analyzed using Kruskal–Wallis followed by a Dunn's test. All statistical analyses were performed in RStudio 2023.06.0 [19].

3. Results

3.1. Growth. There were significant differences in growth rates among treatments in trial 1, as the larvae reared with the 1 mL injector and the 4-inch airlift grew significantly faster than those reared in control tanks (Table 1). Larvae from 1 mL injector treatments were significantly larger than control larvae from 8 to 12 dpf (Table 2). In trial 2, control larvae grew faster than those reared in small-scale systems, regardless of aeration treatment. Larvae from control systems were larger than those in the 5 mL injector and zero aeration microcosms at 6 dpf. Control larvae remained larger than those with zero aeration at 8 dpf. By 10 dpf, control larvae were larger than larvae from all other treatments. On average, larvae grew at $10.4 \pm 1.94 \mu\text{m day}^{-1}$ in trial 1 and $11.2 \pm 1.85 \mu\text{m day}^{-1}$ in trial 2.

3.2. Survival. As the stocking density for all tanks was reduced to 5 larvae mL^{-1} on 2 dpf, analysis of survival began at 4 dpf. There were no significant differences among treatments in trial 1 (Figure 2). In trial 2, there was only a significant difference in survival at 4 dpf (Figure 3), with the 1 mL injector having higher survival than the large-scale controls.

3.3. Days to First Eyed Larvae. There were no significant differences among treatments for days to first eyed larvae in trials 1 or 2 (Table 1). The average time to observe eyed larvae was nearly identical between trials, equaling 12.8 ± 0.7 dpf and 12.8 ± 0.6 dpf in trials 1 and 2, respectively.

3.4. Number of Eyed Larvae. There were no significant differences among treatments in the total number of eyed larvae

TABLE 2: Larval size at each sampling day, reported as days post fertilization (dpf) through 10 dpf, immediately prior to the removal of eyed larvae.

Treatment	Larval age (days post fertilization, dpf)			
	4 dpf	6 dpf	8 dpf	10 dpf
Trial 1				
Control	95.0 ± 1.80	111 ± 8.25	114 ± 7.57 ^b	123 ± 10.0 ^b
2-inch airlift	95.8 ± 2.36	112 ± 3.01	128 ± 12.8 ^{ab}	149 ± 13.0 ^{ab}
4-inch airlift	98.2 ± 1.15	112 ± 7.25	135 ± 5.51 ^{ab}	158 ± 19.4 ^{ab}
1 mL air injector	96.3 ± 4.07	115 ± 5.22	143 ± 4.44 ^a	169 ± 13.4 ^a
<i>p Value</i>	0.525	0.916	0.016	0.022
Trial 2				
Control	93.0 ± 4.42	120 ± 3.62 ^a	147 ± 7.01 ^a	205 ± 17.3 ^a
1 mL air injector	86.3 ± 1.15	113 ± 2.65 ^{ab}	135 ± 4.80 ^{ab}	169 ± 3.62 ^b
5 mL air injector	87.0 ± 4.58	108 ± 5.03 ^b	141 ± 4.92 ^{ab}	170 ± 7.86 ^b
Zero aeration	87.6 ± 1.95	107 ± 2.52 ^b	129 ± 6.38 ^b	169 ± 6.17 ^b
<i>p Value</i>	0.141	0.007	0.027	0.006

Bold *p Values* indicate significant differences among treatments ($p < 0.05$), with pairwise comparison results denoted using superscripts.

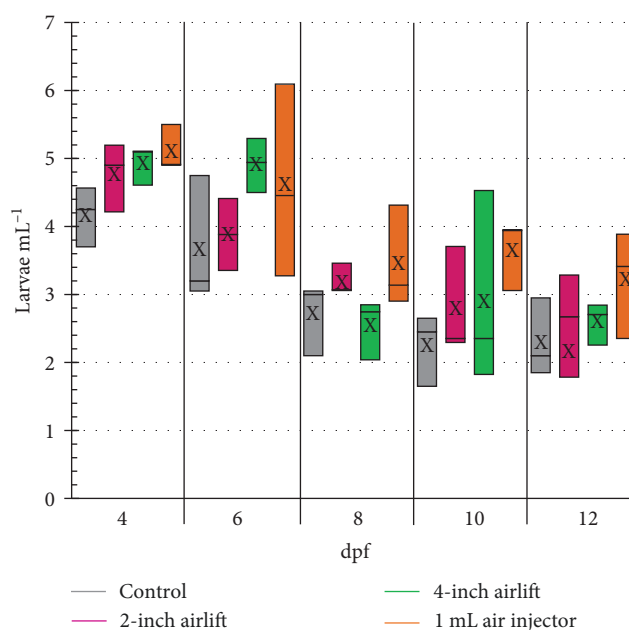


FIGURE 2: Larval survival (larvae mL⁻¹) in trial 1 by aeration type until appearance of first eyed larvae. The upper and lower boundaries of the box represent the highest and lowest survival among replicates, respectively. The center line indicates the survival of the third replicate. The average survival is represented by an X. There were no significant differences in survival among treatments. Manual reductions of larval density occurred at 6 dpf (reduced to 4 larvae mL⁻¹) and 8 dpf (removed larvae less than 75 μm).

after 14 dpf in trial 1 (Table 1). Despite the average of eyed larvae being 10 times higher in the 1 mL injector treatment compared to the controls, there was high variability across replicates within this treatment. In trial 2, however, the controls had significantly more eyed larvae at the end of the experiment, having on average almost four times as many eyed larvae as the microcosm systems.

3.5. Water Quality. In trials 1 and 2, the temperature of the tanks in the morning prior to water change was significantly higher in the controls than the microcosms (Table 3). Immediately following water changes, temperatures were similar

across treatments (*Supplementary Table 1*) and remained similar during PM readings. Overnight changes in temperature demonstrated the same pattern in both trials with microcosms dropping ~2°C in trial 1 and ~3°C in trial 2 versus only ~1°C in controls (Figure 4; $p < 0.001$).

There were no differences in salinity across treatments in trial 1 regardless of the time point measured (AM, post-water change or PM). In addition, there was no difference in overnight change in salinity during trial 1. In trial 2, the average salinity throughout the day remained the same; however, the microcosms had a small but significant change in salinity overnight ($p < 0.001$).

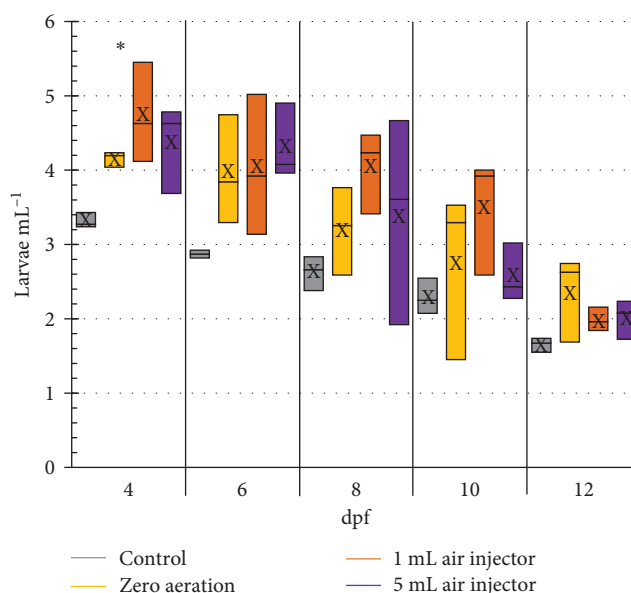


FIGURE 3: Larval survival (larvae mL⁻¹) in trial 2 by aeration type until appearance of first eyed larvae. The upper and lower boundaries of the box represent the highest and lowest survival among replicates, respectively. The center line indicates the survival of the third replicate. The average survival is represented by an X. The asterisk at 4 dpf represents a significant difference between the controls and the 1 mL air injector. Manual reductions of larval density occurred at 6 dpf (reduced to 4 larvae mL⁻¹) and 8 dpf (removed larvae less than 75 μm).

TABLE 3: Water parameters measured in controls and microcosms throughout the 14-day trials.

	Temperature (°C)		Salinity (ppt)		Dissolved oxygen (mg L ⁻¹)	
	AM	PM	AM	PM	AM	PM
Trial 1						
Control	27.2 ± 1.00 ^a	28.0 ± 0.78	21.0 ± 1.82 ^a	20.8 ± 1.84	—	—
2-inch airlift	26.6 ± 1.26 ^b	28.2 ± 1.05	21.0 ± 1.87 ^a	20.8 ± 1.86	—	—
4-inch airlift	26.6 ± 1.26 ^b	28.2 ± 1.04	21.0 ± 1.87 ^a	20.8 ± 1.85	—	—
1 mL air injector	26.6 ± 1.27 ^b	28.1 ± 1.06	20.9 ± 1.90 ^a	20.8 ± 1.88	—	—
<i>p Value</i>	<0.001	0.554	0.041*	0.470	—	—
Trial 2						
Control	26.9 ± 0.96 ^a	28.0 ± 0.99	19.4 ± 1.67	19.1 ± 1.67	5.78 ± 0.71 ^b	6.20 ± 0.29 ^b
1 mL air injector	25.6 ± 1.40 ^b	28.9 ± 1.49	19.3 ± 2.06	19.1 ± 1.93	7.21 ± 0.41 ^a	6.63 ± 0.23 ^a
5 mL air injector	25.6 ± 1.38 ^b	29.0 ± 1.40	19.3 ± 2.07	19.1 ± 1.93	7.18 ± 0.38 ^a	6.64 ± 0.21 ^a
Zero aeration	25.8 ± 1.39 ^b	29.1 ± 1.44	19.3 ± 2.07	19.1 ± 1.93	5.50 ± 1.00 ^c	6.02 ± 0.59 ^c
<i>p Value</i>	<0.001	0.058	0.860	0.957	<0.001	<0.001

Dissolved oxygen was only measured during trial 2. Bold *p Values* indicate significant differences among treatments ($p < 0.05$), with pairwise comparison results denoted using superscripts. *Pairwise comparisons did not detect significant differences among treatments ($p > 0.05$).

Dissolved oxygen was only measured during trial 2. Oxygen levels were significantly higher in the 1- and 5- mL injectors than in the control or zero aeration treatments in the morning. Despite these levels being similar immediately following water changes, the air injector treatments had higher dissolved oxygen than the other treatments in the PM. Overnight, the oxygen in injector treatments increased by an average of 0.44 mg L⁻¹, whereas the control and zero aeration treatments decreased by about 0.56 mg L⁻¹ ($p < 0.001$).

4. Discussion

Results from this study indicate that larvae can be successfully cultured in small-scale systems using algae concentrate. The

performance of larvae reared in these small systems was comparable to those reared in large-scale systems, with eyed larvae appearing between 12 and 13 dpf. The type of aeration used in small-scale systems has little impact on larval performance.

Early in the larval cycle of trial 2, the 1 mL injector microcosms had greater survival than the large-scale systems, but, following reductions in stocking density and size grading that occurred throughout the 2-week trial, these differences were no longer detectable at 6 dpf. Invertebrate larvae may be damaged in higher aeration culture systems resulting in greater mortality, particularly in smaller systems [20–22]. In scallops (*Pecten maximus*), a species for which aeration is particularly detrimental in small-scale systems, aeration stress impacts energy storage, metabolism, and immune function, ultimately

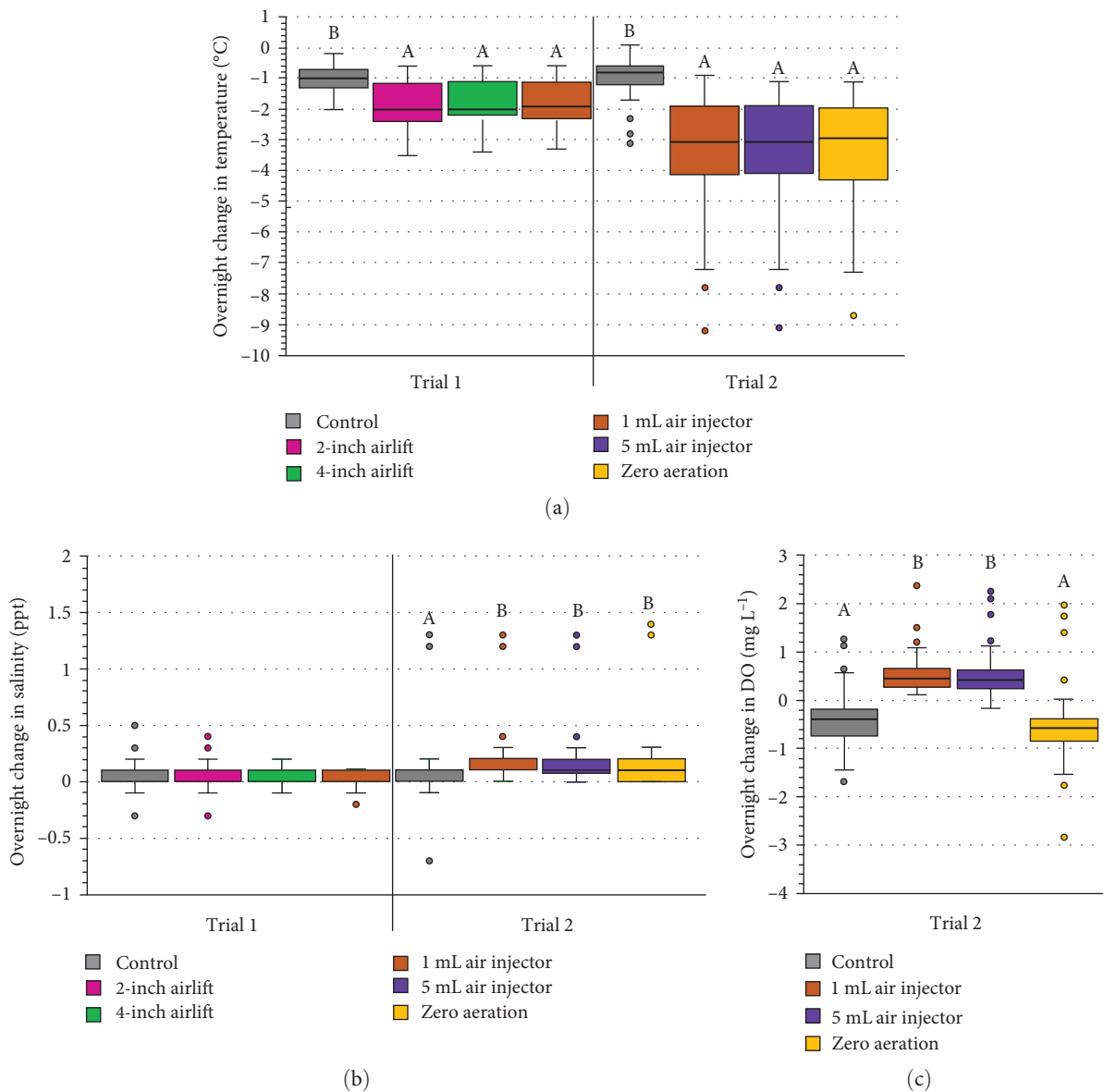


FIGURE 4: Average overnight change in water quality parameters for (a) temperature, (b) salinity, and (c) dissolved oxygen. Dissolved oxygen was not measured in trial 1. Letters denote significant differences among treatments.

reducing growth and leading to mortalities [22]. Some studies report improved survival in invertebrate larvae subjected to high aeration [23]. Differences in aeration also impact larval feeding behaviors [24, 25] and distribution of algae concentrate [26], and so may alter contact rates between larvae and algae. Varied access to feed could influence nutrition, growth, and survival. Regardless, these differences were only present early in larval rearing and did not negatively impact the number of eyed larvae harvested from the systems.

Control tanks maintained higher overnight water temperatures than small-scale systems. These warmer water temperatures may alter microbial community dynamics within the system [27, 28]. These community shifts would likely be most pronounced in the early days of the trial where water was only changed every other day, giving the bacteria a relatively long period for replication. At these times, larvae would

be at the highest stocking densities and physiologically at their most vulnerable [29, 30]. Alterations in oyster-associated bacterial communities are commonly reported during disease and mortality events [27, 31–33]. Interestingly, the larvae in small-scale airlift systems had similar mortality rates to controls. We noticed that these airlifts had the tendency to accumulate algae and, due to their larger surface area, may have provided an ideal surface for biofilm proliferation. In addition, there was a pinkish biofilm that formed in the submerged end of the airlines of these systems. This biofilm appeared at 4 dpf, peaked at 8 dpf, and was not observed after 10 dpf. A pinkish biofilm has been associated with mortality events in other invertebrates [34–36] and may support the hypothesis of negative bacterial interactions in these small-scale airlift systems; however, additional experiments are required to test this. The algal accumulation and pink biofilm

were not detected in large-scale systems. As the airlift components for small-scale systems were more expensive, more difficult to clean, and provided greater potential for microbial concerns when compared to the injector and zero aeration treatments, we do not recommend their use in these microcosms.

Regardless of the reason for the differences in survival in the control systems, these large-scale tanks produced more eyed larvae by the end of the second 14-day trial than the small-scale systems. Control larvae were significantly larger than all microcosm larvae by 10 dpf, just prior to the appearance of first eyed larvae. It is possible that the control larvae reached larger sizes due to reduced competition for resources resulting from early mortality, leading to faster growth and more larvae developing eye spots during the experimental period.

Overall growth rates were higher in trial 2 than in trial 1, with control systems producing nearly four times as many eyed larvae after 14 days as the small-scale systems. It should be noted that the oyster larvae used for these trials came from two different broodstock lines. We cannot rule out the possibility that differences in development between trials were due to variation in performance between these genetic lines. However, genetic lines do not explain the faster growth in control systems in trial 2. This pattern cannot be attributed to dissolved oxygen, as the values for the controls were similar to those of the zero aeration treatments, or to salinity, which was not significantly different across treatments. Although the daytime temperatures of all systems were similar, the overnight temperatures were lower in the latter trial. Davis and Calabrese [37] reported a doubling in growth of *C. virginica* larvae held at 27.5°C versus those at 22.5°C. The minimum temperature reached in small-scale systems was 22.4°C versus 24.9°C in controls, and the average temperatures in general were lower in microcosms due to these overnight drops, which may explain the slower growth rates.

A faster growth rate in controls at least partially explains the higher number of eyed larvae after the 14-day period, but temperature may have also influenced metamorphosis. Larval setting typically occurs between 26.7°C and 32.2°C, with minimal setting occurring below about 24°C [38]. Control systems never fell below this threshold, whereas small-scale systems were lower than 23°C on the thirteenth night of trial 2, after larvae with eye spots were observed. These low temperatures may have inhibited the metamorphosis of larvae in small-scale systems. In addition, a study by Lutz et al. [39] demonstrated that an increase in temperature from 24°C to 29°C that lasted longer than 3 hr caused a significant increase in setting rates of Eastern oyster larvae, which is a similar temperature shift to what the control systems experienced. Although we cannot definitively link temperature to these differences, it would likely be beneficial to include temperature control in small-scale systems to prevent low temperatures during the cooler months.

In conclusion, small-scale systems were successfully used to rear *C. virginica* larvae using microalgae concentrate. Optimization of microcosms is still required to ensure growth and development is equivalent to large-scale systems, particularly when nighttime temperatures decrease. Because of microalgae accumulation and biofilm formation in airlines of small-scale

airlift systems, these are not recommended. There were no noticeable differences between the injector and zero aeration treatments; however, these trials should be continued past 14 days to determine if these treatments impact final production, including survival following setting. The performance of these systems supports their use in replicated studies to improve oyster larval production.

Data Availability

Datasets are available upon request.

Ethical Approval

The authors confirm that the study was conducted according to ethical policies of the journal, as noted on the journal's author guidelines page.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary Materials

Table S1: average water parameters measured in controls and microcosms following water changes. (*Supplementary Materials*)

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