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Effects of Hypoxia and Anoxia on Larval Settlement, Juvenile Growth, and Juvenile Survival of the Oyster *Crassostrea virginica*

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Abstract. The effects of hypoxia (1.5 mg O_2 l^{-1}, 20% of air saturation) and anoxia (<0.07 mg O_2 l^{-1}, <1% of air saturation) on oyster (*Crassostrea virginica*) larval settlement, juvenile growth, and juvenile survival were studied. Settlement was reduced significantly ($P < 0.05$) in hypoxic treatments, as compared to normoxic treatments (7.3 mg O_2 h^{-1}, 100% of air saturation), and almost no settlement took place in anoxic treatments. After 96 h, 38% and 4% of the larvae placed in hypoxic and anoxic treatments had settled, while 79% settled in normoxic treatments. In the first 144 h after settlement, juveniles in hypoxic treatments grew one third as much as those in normoxic treatments, while juveniles in anoxic treatments did not grow at all. Median mortality times of recently settled juveniles in hypoxic and anoxic treatments were 131 h and 84 h, respectively. We conclude that hypoxic and anoxic waters have potentially detrimental effects on oyster settlement and recruitment.

Introduction

Chesapeake Bay exhibits episodes of oxygen depletion concomitant with seasonal salinity and temperature stratification (Taft *et al.*, 1980; Officer *et al.*, 1984). Oxygen depletion is usually restricted to areas below the pycnocline, but wind stress frequently tilts the pycnocline (Carter *et al.*, 1978; Malone *et al.*, 1986) irrigating shallow areas, where oyster reefs occur, with hypoxic or anoxic water from deeper areas (May, 1973; Sanford et al., 1990). The pycnocline remains tilted for from several hours to two or three days (Malone *et al.*, 1986; Sanford *et al.*, 1987). These events often coincide with the timing of settlement and recruitment of the oyster, *Crassostrea virginica* Gmelin. Reduced settlement or complete settlement failure in localized areas has been attributed to incidents of pycnocline tilting (May, 1973; Abbe, 1986).

Previous studies have demonstrated that tolerance of larval and adult oysters to hypoxia and anoxia increases with developmental stage and body size. Larval stages and juvenile oysters (16 mm height) survive anoxia from hours to days (Widdows *et al.*, 1989), while adult oysters survive periods of unsuitable conditions lasting days or weeks (Galtsoff, 1964; Stickle *et al.*, 1989).

Little is known about the tolerance of settling oyster larvae or recently settled juvenile oysters to hypoxia and anoxia. These stages are pivotal to subsequent recruitment into the population. The objectives of this study, therefore, were to examine the effects of hypoxia and anoxia on settlement of oyster pediveliger larvae and on the growth and survival of recently settled juvenile oysters.

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Materials and Methods

Experimental apparatus

All experiments were performed at 25°C and 21‰ S. Temperature was maintained by controling laboratory temperature and by a circulating water bath in which the experimental chambers were immersed. Three 4-liter flasks of 0.45 μm filtered seawater containing algae (*Isochrysis galbana*) at a concentration of 20,000 cells ml^{-1} were bubbled with air, a mixture of oxygen and nitrogen, or nitrogen. The target oxygen concentrations were 7.3 mg O_2 l^{-1} (100% of air saturation), 1.5 mg O_2 l^{-1} (20% of air saturation), and less than 0.07 mg O_2 l^{-1} (<1% of air saturation). These treatments will be referred to as

normoxia, hypoxia, and anoxia, respectively, although the latter of these conditions is more correctly termed 'micoxia.' Although carbon dioxide was not included in the latter two treatments, pH did not differ significantly ($P < 0.05$, ANOVA) among the three treatments.

Flow-through chambers were constructed to hold larval and juvenile oysters during experimental trials. Each chamber was a 20 ml glass vial closed with a rubber stopper pierced by two 20 gauge needles. Inflow needles were fitted with inverted pipette tips. Outflow needles were cut off even with the bottom of the stoppers and covered with 202 μm Nitex mesh, fine enough to retain pediveliger larvae. Chambers within the same treatment were connected in series as depicted in Figure 1. Stainless steel tubing (1 mm bore) was used throughout. The flow rate through the chambers was about 233 ml h^{-1}, and water residence time in the system was 1 h or less. The flasks of seawater and algae were replaced every 12 h with identical flasks that had been bubbled with the appropriate gases for at least 2 h prior to replacement.

Oxygen concentration at the outflow of each treatment was measured daily with a Strathkelvin Instruments (SI) oxygen sensor (1302) held in a SI microcell (MC100) and coupled to a SI oxygen meter (781) and chart recorder. The oxygen sensor was calibrated daily with air-saturated water and a 0% oxygen solution of sodium borate and crystalline sodium sulfite. Normoxic, hypoxic, and anoxic treatments were consistently maintained at 85–100%, 15–22% and 0–1% of full air saturation, respectively. Outflow concentrations of oxygen did not differ measurably from the inflow concentrations.

Larval settlement experiments

Oyster (*Crassostrea virginica*) pediveliger larvae were reared by the Virginia Institute of Marine Science oyster hatchery at Gloucester Point, Virginia. Oyster shell settlement substrates were conditioned in seawater for 24 h prior to each experiment to develop a settlement-inducing bacterial coating (Fitt *et al.*, 1990). One conditioned oyster shell was placed in each chamber, with the rough side up. Fifty larvae were counted into each chamber with a Drummond Captrol III microdispensor. Only actively swimming larvae were used.

Two chambers were removed daily from each treatment; they were not replaced. Settlement was calculated by expressing the number of settled oyster larvae as a percentage of the total number of larvae introduced into the chamber. The data from the two chambers were pooled as one replicate for that exposure time. The entire larval settlement experiment was repeated five times, resulting in five replicates of normoxic treatments, and three replicates each of hypoxic and anoxic treatments.

Larval settlement data were arcsine transformed, and analysis of variance was performed for each exposure time to test the null hypothesis that the means of the three treatments were equal. For those exposure times in which the null hypothesis was rejected, the Tukey multiple comparison test was performed to determine between which treatment means differences existed (Zar, 1984). Means and standard deviations were back transformed for report in Figure 2.

Juvenile growth and survival experiments

Unless otherwise noted, the term "juvenile" is used in this paper to refer to those oysters 144 h post settlement or less. Oyster pediveliger larvae were allowed to settle on conditioned oyster shells for 2 h just prior to commencement of the experiments. Non-settled larvae were washed off after 2 h. One oyster shell with settled larvae was placed in each chamber, with the rough side up. Two chambers were removed daily from each treatment; they were not replaced. Twenty-five randomly selected live juvenile oysters from each of the two chambers were measured with a compound microscope and an ocular micrometer. Growth was measured as the amount of new shell in the dorsal-ventral axis (height). Mortality was recorded as the proportion of dead juveniles among 50 randomly selected juveniles from each chamber. The data from the two chambers were pooled as one replicate for that exposure/post settlement time. The entire juvenile growth and survival experiment was repeated four times, resulting in four replicates of normoxic treatments, and three replicates each of hypoxic and anoxic treatments.

Growth data were log transformed, and the residuals were examined for homoscedasticity. Analysis of variance was performed to test significance and linearity of the growth regressions. Student's t test was used to determine differences between the normoxic and hypoxic growth regression coefficients and regression elevations (Zar, 1984).

Survival data for juvenile oysters were arcsine transformed. Analysis of variance was performed for each exposure/post settlement time to test the null hypothesis that the means of the three treatments were equal. For those exposure/post settlement times in which the null hypothesis was rejected, the Tukey multiple comparison test was performed to determine between which treatment means differences existed (Zar, 1984). Means and standard deviations were back transformed for report in Figure 4.

Results

Larval settlement

In normoxic treatments at 24 h, the mean settlement of oyster larvae was 38% (Fig. 2). The percentage of settled larvae increased 10–20% per day, and was 79% at 96 h. In the hypoxic treatments, settlement was 18% at 24 h

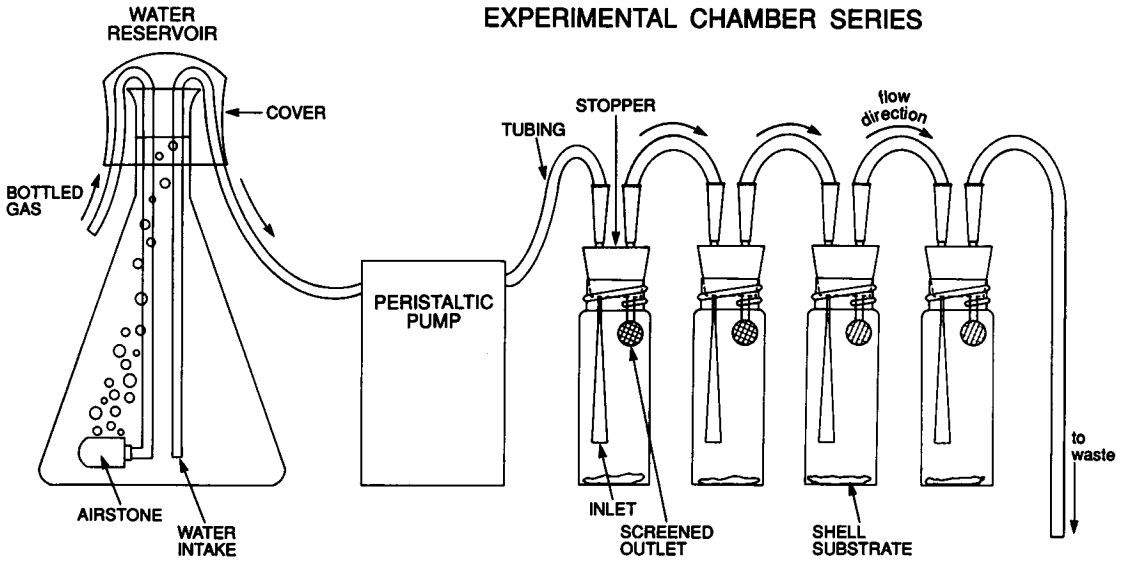

Figure 1. The experimental apparatus. Four chambers of one treatment are shown. Flasks of seawater were bubbled with air, a mix of oxygen and nitrogen, or nitrogen. The equilibrated seawater was pumped through chambers containing settlement substrate and pediveliger larvae or juveniles of the oyster *Crassostrea virginica*. Flow-through chambers were immersed in a circulating water bath of 25°C. (Not drawn to scale.)

and 38% at 48 h. After 48 h, hypoxic treatments had no further settlement. In anoxic treatments, settlement was 4% at 24 h, with no subsequent settlement. At 24 h, anoxic and normoxic treatment means were significantly different ($P < 0.05$), and at 48 h, the anoxic treatment mean was significantly different ($P < 0.05$) from both the hypoxic and normoxic treatment means. At 72 and 96 h, all three treatment means were significantly different ($P < 0.05$) from each other.

Juvenile growth

Regressions of log transformed juvenile oyster growth data from normoxic and hypoxic treatments were linear and significant. The regression coefficients of the normoxic and hypoxic treatments were not significantly different; however, the regression elevations were significantly different ($P < 0.05$) from each other (Fig. 3). Juveniles in the normoxic treatments grew over 255 μm of new shell

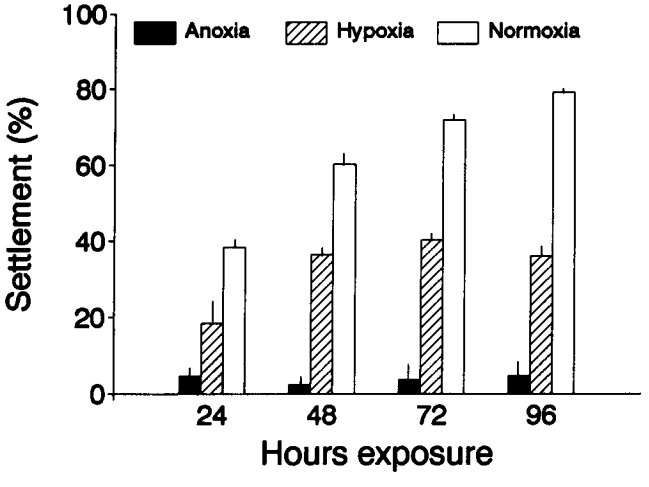

Figure 2. Relation between percentage settlement of oyster (*Crassostrea virginica*) pediveliger larvae and duration of normoxic (7.3 mg O_2 l^{-1}), hypoxic (1.5 mg O_2 l^{-1}), and anoxic (<0.07 mg O_2 l^{-1}) treatments. (Means + SD; normoxia n = 5; hypoxia n = 3; anoxia n = 3.)

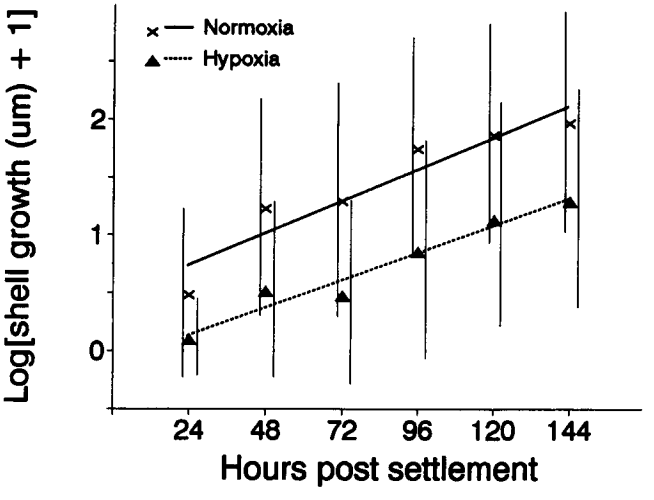

Figure 3. Log of growth of *Crassostrea virginica* juveniles (initial shell height 290 μm) in normoxic (7.3 mg O_2 l^{-1}), hypoxic (1.5 mg O_2 l^{-1}), and anoxic (<0.07 mg O_2 l^{-1}) treatments in relation to hours post settlement. (Means ± SD; normoxia n = 175 for each mean marker; hypoxia n = 125 for each mean marker.)

in 144 h, nearly doubling in length. Juveniles in hypoxic treatments grew only 77 μm of new shell in 144 h, approximately one third as much as those in normoxic treatments. Juveniles in anoxic treatments did not increase in shell height.

Juvenile survival

Juvenile oyster survival was similar in all three treatments for the first 72 h (Fig. 4). At 96 h and 120 h, the anoxic treatment mean was significantly different ($P < 0.05$) from both hypoxic and normoxic treatment means. All three treatment means were significantly different ($P < 0.05$) from each other at 144 h. Juveniles in the anoxic treatments had a median mortality time (time to 50% mortality) of 84 h. Mortality of juveniles in anoxic treatments was 100% by 144 h. Juveniles in the hypoxic treatments had a median mortality time of 131 h. Normoxic treatments, in contrast, had a mean of only 13% mortality at 144 h.

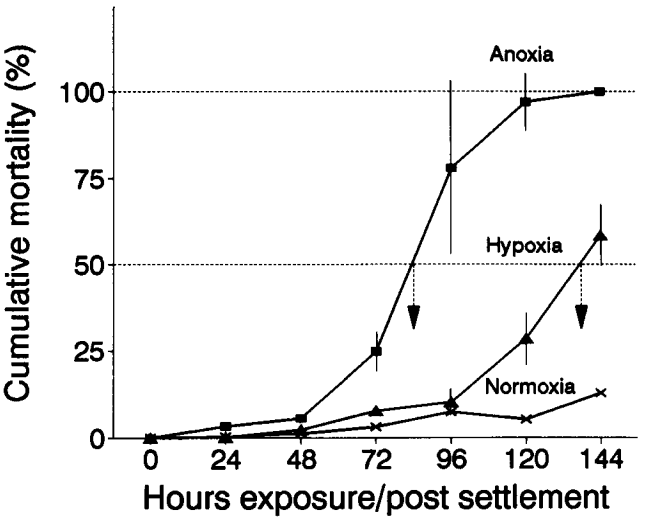

Figure 4. Relation between cumulative mortality of *Crassostrea virginica* juveniles and duration of normoxic (7.3 mg O_2 l^{-1}), hypoxic (1.5 mg O_2 l^{-1}), and anoxic (<0.07 mg O_2 l^{-1}) treatments. Arrows indicate median mortality times. Where no standard deviation is shown, the standard deviation is smaller than the mean marker. (Means ± SD; normoxia n = 4; hypoxia n = 3; anoxia n = 3)

Discussion

Under hypoxic and anoxic conditions, oyster pediveliger larvae significantly reduce energetically costly activities, thereby reducing total metabolism and oxygen requirements (Widdows *et al.*, 1989). The results of this study indicate that settlement is another costly activity that oyster pediveliger larvae avoid when in oxygen-limiting environments.

In a recent paper on the effects of hypoxia and anoxia on *Mytilus edulis* larvae, Wang and Widdows (1991) report that moderate hypoxia has little effect on larval settlement. Settlement of mussel pediveliger larvae onto adult byssus filaments is approximately 12% after two days in conditions of 8.2 mg O_2 l^{-1} (20.0 kPa pO_2, 98% of air saturation at 15°C and 31‰), 2.4 mg O_2 l^{-1} (5.91 kPa pO_2, 29% of air saturation), or 1.3 mg O_2 l^{-1} (3.16 kPa pO_2, 15% of air saturation). An oxygen concentration of 0.6 mg O_2 l^{-1} (1.38 kPa pO_2, 7% of air saturation) shows 1% settlement. Settlement of *C. virginica* appears to be more sensitive to moderate hypoxia than mussel settlement. While settlement of mussel larvae is unchanged in treatments of 8.2 mg O_2 l^{-1} down to 1.3 mg O_2 l^{-1} (Wang and Widdows, 1991), oyster larval settlement was significantly reduced by oxygen concentrations of 1.5 mg O_2 l^{-1} or less. The estimated oxygen concentration at which settlement after two days is 50% of that in normoxic treatments is 0.9 mg O_2 l^{-1} (10% of air saturation) for mussel larvae (Wang and Widdows, 1991) compared to 1.4 mg O_2 l^{-1} (20% of air saturation) for oyster larvae. While oysters are entirely sessile once they have settled, post larval mussels migrate repeatedly before arriving at a final settlement site (Lane *et al.*, 1985). Larval mussels, therefore, do not need to be as discriminating as oyster larvae when selecting a suitable settlement habitat.

In other aspects of their physiology, oyster larvae are less sensitive to oxygen deprivation than are mussel larvae. For example, the oxygen concentration at which the respiration rate is 50% of the normoxic rate is 2.3 mg O_2 l^{-1} (5.7 kPa pO_2, 28% of air saturation) for mussel pediveliger larvae (Wang and Widdows, 1991) and 0.9 mg O_2 l^{-1} (2.3 kPa pO_2, 11% of air saturation at 22°C and 12‰) for oyster pediveliger larvae (Widdows *et al.*, 1989). The 10°C difference in temperature at which the mussel (Wang and Widdows, 1991) and oyster (this paper) settlement experiments were performed, and the resulting differences in metabolic rates, may have contributed to the discrepancy observed in oxygen sensitivity of mussel and oyster larval settlement. At 15°C, mussel pediveliger larvae have a normoxic oxygen uptake of 75 pmol O_2 h^{-1} $larva^{-1}$ (Wang and Widdows, 1991), while at 22°C, oyster pediveliger larvae have an oxygen uptake of 400 pmol O_2 h^{-1} $larva^{-1}$ (Widdows *et al.*, 1989).

As discussed earlier, pediveliger larvae reduce energetically costly activities during hypoxic exposure, such as ingestion, digestion, and growth, thereby reducing oxygen demand. Under hypoxic conditions, there is a marked decline in the proportion of pediveliger larvae feeding and in ingestion rates (Widdows *et al.*, 1989). Mussel pediveliger larvae also exhibit depressed feeding rates and growth in hypoxic conditions (Wang and Widdows, 1991). The reduction of juvenile oyster growth in hypoxic treatments and complete lack of growth in anoxic treatments

observed in this study may have resulted from a cessation of feeding.

In this study, juvenile oysters had a median mortality time of 84 h in anoxia. This indicates that, like oyster larvae and adults, recently settled juvenile oysters are capable of anaerobic metabolism. Widdows *et al.* (1989) report median mortality times in anoxia of 11, 18, and 51 h for oyster prodissoconch, veliconch, and pediveliger larvae, and 150 h for juveniles 16 mm in shell height. The data for recently settled juveniles are consistent with the trend of increasing anoxic tolerance with developmental stage and body size. The increased median survival time in later stages is associated with an ability to reduce energy use, measured as heat dissipation, under anoxic conditions (Widdows *et al.*, 1989). The degree of heat dissipation reduction by recently settled juvenile oysters in anoxia is expected to be between that of the pediveliger larvae and 16 mm juveniles studied by Widdows *et al.* (1989).

Further studies on feeding, heat dissipation, and oxygen uptake are required to understand more clearly the effects of anoxia and hypoxia on settling pediveliger larvae and recently settled juvenile oysters. The present study does demonstrate that hypoxic and anoxic conditions have detrimental effects on larval settlement, juvenile growth, and juvenile survival. Oyster distribution may be influenced by anoxia and hypoxia, especially in those areas that experience prolonged (longer than 48 to 72 h) or severe (anoxic) pycnocline tilt events. Pycnocline tilt events may control recruitment into the adult population directly, because of larval settlement failure and juvenile mortality, and indirectly, because of a reduction in the growth rate of juveniles.

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