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EFFECT OF DECREASING OXYGEN TENSION ON SWIMMING RATE OF *CRASSOSTREA VIRGINICA* (GMELIN, 1791) LARVAE

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ABSTRACT Four sizes of *Crassostrea virginica* Gmelin larvae (mean lengths 76.8, 118.1, 139.7 and 290.2 μm) were exposed to stepwise decreases in oxygen concentration from 100% saturation (5.38 ml/l at 22°C and 22 ppt salinity) to as low as 10% saturation and their swimming rates (net vertical movement per unit time) were recorded at each oxygen concentration. No cessation of swimming was observed and in only two conditions, that of 76.8 μm larvae at 10% saturation and 290.2 μm larvae at 21% saturation, was swimming rate significantly lower than that of the same size larvae at full saturation.

KEY WORDS: swimming, *Crassostrea virginica*, larvae, oyster, oxygen

INTRODUCTION

The Chesapeake Bay and its tributary subestuaries experience seasonal stratification in terms of density, salinity, temperature and dissolved oxygen content. Examination of seasonal hypoxia and anoxia has been the focus of much recent and continuing work (Mackiernan 1987). The seasonal occurrence of stratification coincides with or partially overlaps the period of spawning and settlement of the oyster *Crassostrea virginica* Gmelin. Although the spatial occurrence of hypoxia or anoxia is usually restricted to deeper waters, the seiching of deeper waters due to wind stress periodically results in irrigation of the shallower areas, where oyster reefs abound, with hypoxic or anoxic water. The present consensus is that bivalve larvae employ depth regulation to effect their retention in shallow, stratified estuaries (Mann 1986). The possibility therefore exists that larval stages of the oyster are subjected to stress of hypoxia or anoxia in the Chesapeake Bay during their planktonic existence if hypoxic conditions prevail in the deeper, more saline, upstream-flowing waters that are considered integral to the mechanism of larval retention. What, then, is the behavioural response of oyster larvae to decreasing oxygen tensions similar to that experienced in descending from surface waters to deeper, hypoxic strata? Would swimming behaviour result in avoidance of all but near-saturated water with resultant isolation of larvae in surface, seaward flowing water and their eventual loss from the estuarine system, or would hypoxia result in valve closure and loss to the benthos due to sinking, or would some intermediary response be evident? With these options in mind, the following study examined the swimming response of various developmental stages of oyster larvae to stepwise decreases in oxygen tension.

MATERIALS AND METHODS

Oyster, *Crassostrea virginica* Gmelin, larvae at various stages of development were obtained from the Virginia Institute of Marine Science (VIMS) oyster hatchery. Details of oyster spawning procedure and larval culture were similar to the techniques previously described for the hard clam, *Mercenaria mercenaria* L., by Castagna and Kraeuter (1981). Ripe oysters were spawned by thermal stimulation, the resultant eggs fertilized and the cultures maintained in water originating from the York River at Gloucester Point. Experimental larvae therefore originated from several parents rather than a single male-female cross. Larvae were cultured in 1000l tanks and the water changed at intervals of two days. At each water change larvae were fed with additions of the flagellate *Isochrysis galbana* Parke. No attempt was made to control the salinity of the culture water, which typically varies in the range 15–22 ppt at the hatchery site. When culture salinity differed from the desired experimental salinity larvae were acclimated to the latter by daily water changes with salinity adjustment not exceeding 2 ppt/day. Larvae of first-shelled (straight hinge), mid development (umbo) and competent-to-metamorphose (pediveliger) stages were used in experiments. Appropriate size ranges of larvae were obtained by selective sieving on nylon mesh screens. All larvae of one size class were from the same culture. Two cultures from the same parental broodstock were used to provide the four size classes examined.

All experiments were effected at 22 ppt salinity and 22°C, water temperature being maintained by control of the laboratory air temperature. All observations of larval swimming were made in vertically oriented, square cross-section, borosilicate tubing (Wale Apparatus, Hellertown, PA) measuring 30 cm H × 6 mmL × 6 mmW internal dimensions (approximately 10.8 ml volume). The tube walls were optically flat and allowed both direct observation of larval swimming and video recording. Both upper and lower ends of the tube were covered with 20 μm nylon mesh to retain larvae. Over each mesh was placed butyl rubber or Fisher brand C-flex tubing (of low porosity to

oxygen) attached to a valve. The glass tube thus formed a chamber that could be sealed at both the top and bottom. The top valve was connected to a variable speed peristaltic pump (Buchler). The bottom valve was connected to a conical flask in which sea water, at the experimental temperature and salinity, was bubbled with an appropriate mixture of nitrogen and air to obtain stable oxygen concentrations of less than 100% of saturation. Oxygen tension in the conical flask was measured with either a Radiometer or Strathkelvin oxygen electrode connected to a Strathkelvin 781b amplifier/meter. The electrode was calibrated daily.

The experimental procedure started with closing the bottom valve, temporary removal of the top valve and associated mesh, partial filling of the glass tube with air-saturated sea water, gentle addition of larvae and air-saturated sea water using a Pasteur type pipette to fill the tube and replacement of the top mesh and valve. Between 30 and 150 larvae, equivalent to concentrations of 3 and 15 larvae/ml when uniformly dispersed, were used with numbers increasing at smaller sizes. Larvae were allowed to recover for approximately 30 minutes, recovery being recognized by continuous active swimming. Throughout this period larvae were continually observed. Following acclimation video recordings were made of swimming activity for later determination of individual larval swimming rate. After a period of exposure at saturation, larvae were exposed to stepwise decreases in oxygen tension. These were accomplished by opening both the bottom and top valves and gently aspirating water through the tube from the conical flask using the peristaltic pump. Larvae were retained by the mesh, but a complete flushing (checked by a previous dye study) of water was effected in a 2–3 minute period. The valves were then closed, the pump turned off and the larvae allowed to re-equilibrate for approximately 5 minutes, still under constant observation, before further measurements of swimming rate was made.

The experiment employed stepwise decreases in oxygen tension from saturation to lower values (given in Table 1). A typical experimental protocol required 20–25 minutes at each oxygen tension before a subsequent further decrease. An experiment examining five different oxygen tensions required approximately two hours to complete. On termination of the experiment, both valves were opened, the larvae were drained through the bottom valve, retained on a 53 μm mesh, transferred to a glass shell vial, fixed in 5% v/v buffered formalin and subsequently measured to obtain a mean individual length (maximum dimension parallel to the hinge line) using a compound microscope equipped with a calibrated ocular micrometer.

Recordings for estimation of swimming rate were made with the system described in Mann (1988). A high resolution, IR sensitive video camera (Dage-MTI SC65S with Ultricon phototube: Eastern Microscope Co., Raleigh, NC) was mounted on a vertically travelling stage (Velmex, Inc., E. Bloomfield, NY). The stage was driven by a Bodine S41 motor and Minarik SL-15 speed control allowing a variable speed traverse from 0.1–10 mm/sec—encompassing larval swimming speeds as recorded in the literature (see review by Mann 1986). General observation of all larvae in the tube was facilitated by movement of the camera on the stage; however, measurements of swimming rate were made with the camera fixed. Illumination for macro-video recording was facilitated by attaching a fiber optic ring light (Fiber Optic Specialties, Inc., Peabody, MA, model LS81A fiber optic with FA-83 filter holder) to the camera lens (50 mm Series E Nikon attached to a Nikon PB-6 bellows and video C-mount). Even though earlier experiments (Mann, unpublished data) had failed to demonstrate a response by larvae to intense, orientated white light, this potential artifact was eliminated by inserting a 695 or 850 nm long pass filter (Oriel Corp., Stratford, CT) in the filter holder and recording under low intensities of essentially IR light. Room lighting was maintained at minimal levels throughout the experiment. Video recording (Panasonic NV-8950 recorder and Panasonic WV-5410 monitor) at such low light levels is not problematic in that the Dage SC65S camera has high sensitivity to light wavelengths up to 1200 nm and operates optimally at an intensity of 3.7×10^{-3} $\mu W.cm^{-2}$ (approximately 0.1 foot candles).

Prior to each experiment a calibrated (in mm) plexiglass ruler was suspended in the borosilicate tube, equidistant between its front and back (with respect to the "view" of the camera) walls. The camera was focussed on the ruler and its magnification adjusted, using the bellows, until a distance of approximately 4 mm on the ruler filled the vertical displacement on the monitor screen. The camera focus and magnification were then fixed and a recording made of the ruler scale on the video tape together with a time and date overlay (Panasonic WJ-810 time-date generator). An audio commentary describing larvae to be used, proposed oxygen exposure regime and other relevant experimental details were also included on the videotape. The ruler scale provided the basis for all subsequent measurements of larval swimming rate from that experiment's recordings. Measurements of individual larval swimming speed were not made during the experiment, but were recorded from replay of the videotapes. A grid, corresponding to the aforementioned ruler calibration, was temporarily fixed to the video monitor and the video tape replayed at reduced speed. The field of observation corresponds to a volume of approximately 0.13 ml which typically contained 1–4 larvae at any one time during recording. The vertical movement of individual larvae across fixed intervals of the grid was timed using the time elapsed recording on the video tape. From individual rates a measurement of mean rate of net vertical movement for each size of larva examined was thus obtained. Only one videotape was used per experiment to eliminate possible confusion in subsequent data analysis.

TABLE 1.

Swimming rate (net vertical movement per unit time in mm/sec) of oyster larvae at various concentrations of dissolved oxygen. ml/l values calculated from % saturation using Table 4 of Carpenter (1966) assuming salinity = 0.03 + 1.805 × chlorinity (Sverdrup et al. 1942).

Age	Length	S.D.		D.O.		Swimming Rate mm/sec					
Days	µm	µm	n	% sat	ml/l	Mean	S.D.	Min	Max	95% Interval	n
2	76.8	2.1	30	100	5.38	0.98	0.25	0.63	1.43	0.80–1.16	10
				77	4.14	0.85	0.51	0.27	2.17	0.48–1.21	10
				45	2.42	0.99	0.36	0.45	1.47	0.73–1.25	10
6	118.1	10.6	32	100	5.38	1.48	0.67	0.27	2.27	1.01–1.96	10
				56	3.01	1.12	0.44	0.45	2.00	0.80–1.44	10
				36	1.94	1.21	0.73	0.35	2.50	0.69–1.73	10
				28	1.50	1.15	0.69	0.35	2.50	0.66–1.65	10
				10	0.54	0.64	0.30	0.25	1.10	0.42–0.85	10
10	139.7	21.7	30	100	5.38	1.79	0.79	0.63	2.94	1.23–2.36	10
				50	2.69	1.94	0.88	0.50	3.13	1.31–2.57	10
				22	1.18	2.22	1.18	0.94	4.17	1.37–30.6	10
13	290.2	28.2	30	100	5.38	3.10	1.35	1.32	5.00	2.14–4.07	10
				45	2.45	1.93	0.75	1.14	3.13	1.40–2.47	10
				21	1.13	1.36	0.42	0.86	2.38	1.06–1.66	10

RESULTS

Table 1 summarizes data on age and length of larvae examined and their respective swimming rates under various concentrations of dissolved oxygen. Mean net vertical swimming rates vary in the range of 0.64–3.10 mm/sec. A series of one-way analyses of variance were performed comparing swimming rates at each oxygen concentration at each size. Significant differences with decreasing oxygen were observed at 118.1 µm length, where the swimming rate at 10% of saturation was lower ($P < 0.05$) than at saturation. At 290.2 µm length (pediveliger larvae) the swimming rate at 21% of saturation was lower ($P < 0.01$) than at saturation. With these exceptions decreasing oxygen concentration was not accompanied by a significant decrease in mean swimming rate within the time course of the experiment. Comparisons of mean swimming rate of different sizes of larvae are complicated by the fact that, with the exception of values recorded at saturation, oxygen concentrations and immediately prior oxygen environment are not identical. First-shelled veliger or D larvae at 76.8 µm have statistically significant lower swimming rates at saturation than larvae with shell lengths geater than 139.7 µm. Pediveliger larvae of 290.2 µm length swim at significantly faster rates than either 76.8 or 118.1 µm length larvae at saturation.

DISCUSSION

The most significant findings of this study are that *Crassostrea virginica* larvae do not cease swimming as oxygen concentration decreases and that a statistically significant decrease in swimming rate is not observed until larvae are exposed to the lowest oxygen concentrations examined, even when exposure periods approach 20–25 minutes at each concentration and cumulative exposure to increasing levels of hypoxia approaches two hours. Previous studies with *C. virginica* larvae suggest a predominantly lipid-protein based, aerobic energy metabolism (Gallager et al. 1986) similar to that of shipworm larvae at normoxia (Mann & Gallager 1985). The present observations suggest that aerobic metabolism can be maintained at hypoxia due to the large surface to volume ratio of all stages of veliger larvae examined, that oxygen requirements of the velar cells responsible for swimming can be satisfied in that diffusion pathways to them are short, and/or that some limited capability for anaerobiosis is present. Recently Widdows et al. (1989) examined heat production, oxygen consumption and feeding of *C. virginica* larvae under prolonged hypoxia and anoxia. They concluded that such larvae have limited capability to function anaerobically under hypoxia, as indicated by both feeding and activity observation. They also recorded a notable difference in response to prolonged anoxia and hypoxia exposure stress by first-shelled and pediveliger larvae in that the former maintain activity under stress (essentially an avoidance response), but eventually succumb within a few hours, whereas the latter decrease activity within a short period but survive for considerably longer. Given the increase in specific gravity accompanying development from first-shelled to pediveliger larvae, and the contrasting roles in development (dispersal versus seeking metamorphic substrate) of these larval stages these responses are expected. In the present study a similar response is observed for the pediveliger stage, that is a marked reduction in swimming rate at 21% of saturation. With the exception of the lowest oxygen concentration ex-

amined for 118.1 μm larvae the maintenance of activity by larvae in the length range 76.8–139.7 μm under short term hypoxia stress is consistent with the aforementioned observations of maintained activity by Widdows et al. (1989). The exception is notable in that it was recorded at the end of a cumulative hypoxia stress approaching two hours in duration, more consistent with the observation of eventual submission as recorded by Widdows et al. (1989).

Morrison (1971) examined the influence of variable periods of exposure to low oxygen environments on the embryonic and larval development of the hard shell clam *Mercenaria mercenaria*. Eggs developed normally at oxygen concentrations of 0.5 mg/l (7% of saturation at the experimental conditions of 28–30 ppt salinity and 25°C). Larval growth was curtailed at or below 2.4 mg/l (34% of saturation) but proceeded normally above 4.2 mg/l (60% saturation). Larvae were capable of recovering from periods of growth inhibiting hypoxic conditions when subsequently transferred to normoxic conditions. The ability of *C. virginica* larvae to grow under hypoxic stress in a manner comparable to *M. mercenaria* larvae has not been examined but is clearly worthy of study. If such growth capabilities are present then the observation of sustained swimming activity at moderate hypoxia (over 60% of saturation) could be considered normal rather than an avoidance response as suggested earlier. In such an instance the description of an avoidance response should be restricted to sustained swimming activity under hypoxic conditions associated with growth inhibition or cessation.

The methods of estimating swimming rate in the present study represent a significant advance over most previous efforts. The studies of Cragg (1980) and Mann and Wolf (1983) both used travelling microscopes to observe larval swimming. In the latter case, rate and magnitude of vertical movement was recorded, via a ten-turn potentiometer, on a strip chart recorder. A manually operated travelling microscope suffers from a prerequisite for considerable operator dexterity to obtain smooth output traces of larval movement, a need for the operator to estimate (from an eyepiece graticule) the horizontal component of an observed swimming pattern during recording, and a lack of production of a hard record of the observation. Visible light, a prerequisite of direct observation, has been shown to influence the larval swimming of some bivalve species; however, previous experiments described in Mann (1988) in both light proof boxes and under dim laboratory lighting failed to demonstrate any phototactic response in *C. virginica*. In these experiments the travelling microscope was replaced with a fixed video camera operating under appropriate light conditions to eliminate operator variability and provide high quality recordings. It is important to note that the reported values of net vertical movement per unit time differ from absolute swimming speed because the larva swims in a helical pattern; however, this is the ecologically relevant value in terms of rate of depth regulation. Further, the stepwise decreases in oxygen tension without periodic increases were chosen to simulate conditions of larvae gradually sinking from surface waters to deeper, hypoxic water. This was considered to be ecologically more realistic than exposure to a series of randomly chosen concentrations.

Swimming rates reported in Table 1 are comparable to previously reported values for *Crassostrea virginica* larvae by Hidu and Haskin (1978, Fig. 2; 0.83, 1.0 and 1.83–2.33 mm/sec for 80, 160 and 230–270 μm larvae respectively at 25°C and 15–25 ppt salinity) and Mann (1988; 0.37 and 1.02 mm/sec for 75 μm and 157.5 μm larvae respectively at 22°C and 19–22 ppt salinity), for other bivalve veliger larvae including *Ostrea edulis* L. (Cragg & Gruffydd 1975; 1.23 mm/sec for 200–250 μm larvae at 20–21°C and 32–33 ppt salinity), *Teredo bartschi*[1] (Isham & Tierney 1953; 7.7 mm/sec at 20–28°C and unspecified salinity), and a variety of marine invertebrate larvae as reviewed by Mileikovsky (1973).

The range of mean swimming rates recorded in Table 1 (0.64–3.10 mm/sec) correspond to changes in absolute depth of 2.3 and 11.2 meters per hour for continuously swimming larvae. Given the bathymetric range of oyster reefs in the Chesapeake Bay, generally less than six meters in depth, and the shallow nature of the subestuaries of the bay, it is evident that larvae can, through active swimming alone, depth regulate and ensure retention in the proximity of suitable substrate through exploitation of salinity-driven, depth-specific circulation. If oyster larvae sank into deeper hypoxic zones in the Chesapeake Bay then sustained swimming at the aforementioned rates would only be required for intervals of one or two hours to return larvae to normoxic surface waters. This time interval is less than that required to reach a point of submission to hypoxic stress by the first-shelled larvae as reported by Widdows et al. (1989).

The demonstration of unexpectedly high tolerance of oyster larvae to short term hypoxic stress prompts the question of whether larvae are the most susceptible stage of the oyster life cycle to this environmental stress. This may not be so in that small larval stages appear to be able to fulfill aerobic requirements by simple diffusive processes. The same may also be true of a wide variety of marine invertebrate larvae with predominantly lipid-protein based energy metabolism. It is only as size increases, as impermeable external layers (such as shell) develop and the adoption of the sessile benthic form (which cannot escape from hypoxic events) occurs that the ability to supply a major proportion of the metabolic energy from sustained anaerobic activity becomes critical in surviving hypoxic or anoxic stress. Limited data on post settlement changes in gross biochem-

[1]Although described as *Teredo* (*Lyrodus*) *pedicellata* by Isham and Tierney (1953) this species was later shown to be *Teredo bartschi* by Turner and Johnson (1971).

ical composition of the oyster *Ostrea edulis* (see Holland & Spencer 1973) suggest that transition to typically adult anaerobic capabilities, as indicated by an abundance of carbohydrate reserves, may require as long as thirty days. If comparable periods apply to post settlement *Crassostrea virginica* in the Chesapeake Bay then periodic irrigation of shallow oyster reefs by hypoxic water caused by wind driven seiching may be a significant source of stress and mortality, indeed more so than the influence of such events on oyster larvae in the same location.

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