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Oyster (Crassostrea virginica) metamorphosis: Effects of low oxygen

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OYSTER (*CRASSOSTREA VIRGINICA*) *METAMORPHOSIS* - EFFECTS OF LOW OXYGEN

A Dissertation

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Shirley Marie Baker

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Dedicated to the memory of Dr. Robert C. Terwilliger

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ABSTRACT

The objective of my research was to examine the physiology and behavior of metamorphosing oysters, *Crassostrea virginica*, and to investigate the effects of low oxygen stress on metamorphic processes. Specifically, I examined the effects of hypoxia (20% of air saturation) and microxia (< 1% of air saturation) on settlement, survival, growth, morphology, metabolic rate and feeding on post-settlement oysters.

All of the functions I measured were adversely affected by hypoxia and microxia, compared to normoxic controls. Survival times indicate that, like larvae and adults, post-settlement oysters are capable of anaerobic metabolism. The 2 week period following settlement is especially critical to recruitment. Low oxygen conditions increases mortality and have detrimental effects on the development and growth of post-settlement oysters. Oysters have the ability to feed at nearly all stages of settlement and metamorphosis. While hypoxic conditions reduce feeding only in the youngest metamorphosing oysters, microxic conditions affect all ages. Not only does weight-specific metabolism decrease as the oysters grow, but metabolic responses to low oxygen change from relatively oxygen independent to oxygen dependent.

I conclude that oyster distribution may be influenced by low oxygen, especially in those areas that experience prolonged (24-48 h) hypoxia or severe microxic events. Low oxygen events may control recruitment into the adult population directly, because of larval settlement failure and post-settlement mortality, and indirectly, because of reduction in feeding, development rate, and growth of post-settlement oysters.

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THE COLLEGE OF WILLIAM AND MARY IN VIRGINIA

OYSTER (*CRASSOSTREA VIRGINICA*) *METAMORPHOSIS*

- EFFECTS OF LOW OXYGEN

Chapter 1

INTRODUCTION

Nature and Scope of the Problem

The oyster, *Crassostrea virginica* (Gmelin, 1791) has historically been a valuable part of the Chesapeake Bay benthic community, both ecologically (Newell, 1988) and economically (Kirkley, 1987). In recent decades, however, there has been a major decrease in oyster production (Hargis and Haven, 1988). The rapid deterioration of the fishery has been associated with disease, over fishing, low water quality (toxicants, sedimentation, oxygen depletion, etc.), and a decline in recruitment (Haven, 1987). Recruitment into the adult population is regulated by a host of physical and biological factors, including those that affect larval settlement and metamorphosis (Abbe, 1986). Oyster settlement and subsequent metamorphosis occurs between June and September (Haven and Fritz, 1985), coinciding with the occurrence of low water quality in Chesapeake Bay; specifically, hypoxia and microxia (Officer *et al.*, 1984). Oxygen deficient water is usually confined to deeper portions of the bay; however, under specific conditions, hypoxic and microxic water may enter the shallows that oysters inhabit (Breitburg, 1990).

Previous studies on the effects of hypoxia (< 50% of air saturation) and microxia (< 1% of air saturation) on bivalves have focused on tolerance, growth, larval

development, oxygen consumption, total metabolism, and feeding. Tolerance of larval and adult oysters to hypoxia and microxia increases with developmental stage and body size. Larval stages and juvenile oysters (16 mm height) survive microxia from hours to days (Widdows *et al.*, 1989). Exposure to hypoxia causes delayed and abnormal embryonic development and reduced larval growth rates in both the hardshell clam *Mercenaria mercenaria* and the mussel *Mytilus edulis* (Morrison, 1971; Wang and Widdows, 1991). Widdows *et al.* (1989) report that total metabolism in oyster larval stages and juveniles (16 mm height) is maintained by aerobic metabolism down to low oxygen levels (3.1 mg O_2 l^{-1} or 8 kPa pO_2, 38% of air saturation at 22°C and 12 ppt). Late larval stages and juveniles lower their rates of heat dissipation during microxia to a small fraction of the normoxic rate. The process of feeding is interrupted by oxygen deprivation. Under hypoxic conditions there is a marked decline in the proportion of oyster larvae feeding and in ingestion rates (Widdows *et al.*, 1989).

While these studies have addressed the effects of low oxygen conditions on the physiology of larval and adult oysters, no such information is available for the pivotal stages of settlement and metamorphosis. My dissertation research builds upon these previous observations, filling a stage-specific gap in our knowledge of the oyster, *C. virginica*. Understanding the effects of physical conditions

such as hypoxia and microxia, which may influence settlement and metamorphic success, is important in a practical sense in designing future measures to revitalize the rapidly deteriorating oyster fishery, not only in Chesapeake Bay, but elsewhere as well. On a broader perspective, my research contributes to understanding the physical factors limiting settlement and recruitment to the estuarine benthos.

The objective of my research was to examine the physiology and behavior of settling and metamorphosing oysters, *C. virginica*, and to investigate the effect of low oxygen stress on metamorphic processes. Specifically, I examined the effects of hypoxia and microxia on:

1) The settlement success of oyster larvae.
2) The survival of post-settlement oysters.
3) The growth of post-settlement oysters.
4) The morphological changes that take place during metamorphosis.
5) Feeding ability of settling and metamorphosing oysters.
6) Ingestion rates of post-settlement oysters.
7) Total metabolic rates of post-settlement oysters.
8) Respiration rates of post-settlement oysters.

In Chapter 2, I examined the effects of low oxygen on larval settlement success, and post-settlement growth and survival. I tested the following null hypotheses:

A) At each exposure time, mean larval settlement in normoxic, hypoxic, and microxic treatments were equal.

B) Growth regressions were linear and significant.

C) There were no differences in the slopes or elevations of growth regression lines of post-settlement oysters during exposure to oxygen treatments (normoxic, hypoxic, microxic).

D) At each exposure time, mean mortality of post-settlement oysters was equal in normoxic, hypoxic, and microxic treatments.

I examined metamorphosis during continuous and following short-term exposures to low oxygen in Chapter 3. The following null hypotheses were tested:

A) There was no effect of continuous (120 h) oxygen treatment (normoxic, hypoxic, and microxic) on the number of oysters completing metamorphosis to the juvenile phase.

B) There was no effect of oxygen treatment (normoxic, hypoxic, and microxic), length of exposure (1, 2, and 3 d), or interaction of treatment and exposure time, on the number of oysters completing metamorphosis to the juvenile phase by 14 d post-settlement.

C) Growth regressions were significant.

D) There were no differences in the slopes or elevations of growth regression lines of post-settlement oysters following exposure to oxygen treatments for different periods.

In Chapter 4, I examined the feeding rates of post-settlement oysters during exposure to low oxygen. I tested the following null hypothesis:

A) For each size of post-settlement oyster, there were no effects of oxygen treatment (normoxic, hypoxic, and microxic), length of exposure (0-6 h and 18-24 h), or interaction of treatment and exposure time on ingestion rates.

I examined heat dissipation and oxygen uptake rates in Chapter 5. The following null hypotheses were tested:

A) There was no effect of oxygen treatment (normoxic, hypoxic, and microxic), oyster size/metamorphic phase, or interaction of treatment and size, on weight-specific heat dissipation rates of post-settlement oysters.

B) There was no effect of oxygen treatment (normoxic, hypoxic, and microxic), oyster size/metamorphic phase, or interaction of treatment and size on weight-specific oxygen uptake rates of post-settlement oysters.

Review of the Literature

Symbols and units

Over the decades, an enormous number of symbols and units have appeared in the literature to describe the quantity of oxygen and metabolic rates. Oxygen quantities are generally given as pressures or as concentrations. The International System (SI) Unit for the partial pressure of oxygen, pO_2, is the kilopascal (kPa). The SI Unit for the amount-of-substance concentration, cO_2, is μmol O_2 dm^{-3}. Other units used in current literature include mm Hg pO_2, mg O_2 l^{-1}, and ml O_2 l^{-1}. The SI unit for oxygen uptake rate, $\dot{N}$, is μmol O_2 h^{-1}. Again, several units are in use, including $\dot{V}$ in ml O_2 h^{-1}, and mg O_2 h^{-1}. Joules are replacing the calorie as the unit of heat dissipation. Heat dissipation rate, $\dot{Q}$, is measured in mW and is converted to μJ h^{-1}. Oxygen consumption rates can be converted to heat dissipation rates using oxycaloric equivalents, $\Delta_k H_{O2}$, in kJ (mol O_2)$^{-1}$. (For review see: Gnaiger, 1983a.)

Throughout my dissertation, I have used mg O_2 l^{-1}, rather than the SI unit μmol O_2 dm^{-3}, for the amount-of-substance concentration because it is more widely used and recognized. I have, however, used the SI units for oxygen uptake and heat dissipation rates. When the results of other authors are cited for the first time in a chapter, the

published quantity and unit are given in parentheses. Conversion factors for units of amount of oxygen, units of oxygen consumption, and units of heat dissipation are given in Appendix A. A list of symbols with descriptions and units is given in Appendix B.

Hypoxia in Chesapeake Bay

The amount of a gas in water depends on the partial pressure of the gas, the solubility of the gas, the temperature of the water, and the presence of other solutes in the water. At sea level, the atmospheric pressure is 760 mm Hg or 101 kPa. The atmosphere is 20.95% oxygen so the partial pressure of oxygen at sea level is 159 mm Hg or 21.2 kPa pO_2. The solubility of oxygen in, for instance, pure water at 10°C is 0.5 mg O_2 l^{-1} kPa^{-1}. The solubility of oxygen in water decreases with increasing temperature and salinity. The interactions between molecular species in salt solutions account for the "salting out effect". (For review see: Forstner and Gnaiger, 1983.)

The greatest possible oxygen solubility for a given temperature and salinity is that of 100% of air saturation. For instance, at summer temperatures (30°C) and salinities (20 ppt) in Chesapeake Bay, 100% of air saturation is about 6.8 mg O_2 l^{-1}. In my dissertation, the term hypoxia is used to refer to air saturation values of 50% or less. The term anoxia refers to oxygen concentrations of absolute zero. In

most experiments, however, absolute zero is difficult to achieve. In my study, oxygen concentrations of less than 1% of air saturation are referred to as microxia.

Hypoxia and microxia occur annually in the deep waters of Chesapeake Bay, from May through September (Officer *et al.*, 1984; Kuo and Neilson, 1987). During the winter, organic matter from the previous year's plankton bloom settles to the bottom (Taft *et al.*, 1980). When water temperatures increase in the spring, this large pool of biomass begins to decompose (Sellner, 1987; Jonas, 1988). At the same time, spring freshwater runoff increases density stratification and warm freshwater flows out over cool saline water (Taft *et al.*, 1980; Seliger *et al.*, 1985). The interface of the two layers is termed the pycnocline. Such two-layer circulation restricts vertical mixing and thus minimizes oxygen replenishment to bottom waters (Officer *et al.*, 1980). Hypoxia and microxia result when bacterial oxygen consumption exceeds reoxygenation of the deep water (Tuttle *et al.*, 1987).

The temporal and spatial extent of Chesapeake Bay hypoxia has expanded considerably since it was first recorded in 1917 (Sale and Skinner, 1917; Cooper and Brush, 1991). Increased nutrient enrichment from anthropogenic sources has stimulated algal growth and subsequent organic sedimentation. Increased respiration in the deep waters

below the pycnocline consumes a limited supply of oxygen (Fisher and Doyle, 1987).

Generally, hypoxic and microxic conditions are confined to the deeper portions of the Bay and therefore, have little affect on oyster reefs. Under specific conditions, however, oxygen-deficient water may enter the shallows that oysters inhabit (Breitburg, 1990). Prolonged winds, together with tidal currents, cause lateral oscillations of the pycnocline. These oscillations force water onto one shore while water from below the pycnocline advects onto the flanks and into the lower reaches of the tributaries on the other shore (Sellner and Kachur, 1987). Such an event is called "seiching" or "pycnocline tilt". The pycnocline may remain tilted for from several hours to several days (Sanford *et al.*, 1990). Breitburg (1990) examined the incidence of hypoxia at 4 m and 2 m deep sites on an oyster reef during the summers of 1987 and 1988. She found that, at the 4 m site, oxygen dropped below 2.0 mg O_2 l^{-1} (23-29% of air saturation at 20-30°C and 9-16 ppt) on 40% of the days, and below 1.0 mg l^{-1} (12-14% of air saturation) on about 10% of the days. Diel fluctuations in oxygen concentrations, however, were such that dissolved oxygen reached at least 3.4 mg l^{-1} (40-49% of air saturation) at some time during even the most severe days (Breitburg, 1990).

Life cycle of the oyster

The life cycle of *Crassostrea virginica* is depicted in Table I. In Chesapeake Bay, *C. virginica* generally commences spawning when temperatures reach 20°C in early summer, continuing until the fall. The onset and ending of spawning activity and larval settlement, however, is site specific. Oysters in the Maryland portion of Chesapeake Bay typically spawn earlier than those in Virginia. (For review see: Andrews, 1951; Beaven, 1955; Kennedy and Krantz, 1982; Haven and Fritz, 1985; Haven, 1987.) Gametes are released into the water column where fertilization takes place. Zygotes develop into free-swimming trochophore, veliger, and pediveliger larvae. The final larval stage, the "swimming-creeping" stage, was termed the "pediveliger" by Carriker (1956). Pediveliger larvae are approximately 300 μm in diameter and are characterized by a velum, foot, pigmented "eyespots" and gill rudiment. After approximately 18 days of pelagic life, the larvae sink to the bottom and attach to a substrate, often another oyster shell. Following attachment, the larvae metamorphose into the juvenile form. (For review see: Truitt, 1944; Korringa, 1952; Galtsoff, 1964; Andrews, 1979.)

Table I. Life cycle of the oyster, *Crassostrea virginica*. Times are approximate and depend on temperature and other physical factors. Source: Galtsoff, 1964; Chapter 3.

Larval oysters	Time post-fertilization
Zygote	0 h
Trochophore	6 h
Prodissoconch (straight-hinge veliger)	24 h
Veliconch (umbo veliger)	8 d
Pediveliger	18 d
Settlement behavior	18 - 23 d
Searching	
Crawling	
Cementation	

Post-settlement oysters	Time post-settlement
Settler	0 h
Prodissoconch postlarva	18 h
Dissoconch postlarva	29 h
Juvenile	48 h
Adult	1 - 2 years

Larval settlement

Settlement is the term applied to the process of oyster larval attachment. Oyster larvae settle onto hard substrates, preferring oyster shells over other types of substrate (Mann *et al.*, 1990; Michener and Kenny, 1991). Ideal substrates have a film of bacteria but are not excessively fouled (Cole and Knight Jones, 1949; Fitt *et al.*, 1990) and substrates with pits and irregularities attract more pediveliger larvae than do smooth substrates (Prytherch, 1934). Pediveliger larvae exhibit negative phototactic behavior and tend to set in shaded areas or on the underside of substrates (Cole and Knight Jones, 1949; Michener and Kenny, 1991). Larvae exhibit gregarious settlement, being attracted to substrates which already have settled individuals on them (Cole and Knight Jones, 1949; Hidu and Haskin, 1971; Michener and Kenny, 1991).

Oyster larval settlement involves a complex set of behaviors which different authors have divided into various numbers of phases. I prefer to divide settlement behavior into three distinct phases: searching, crawling, and cementation. In the searching phase, larvae swim with the foot extended. When a substrate is encountered, the larva withdraws the velum and starts crawling. The crawling phase begins with fast smooth gliding on the long slender foot with the shell perpendicular to the substrate. At this time the animal's path is fairly straight with curving turns.

Swimming and searching may be resumed. As the crawling phase continues, the foot becomes shorter and broader and the speed slower. Movement becomes jerky as the foot is extended, contracts, and drags the larva along. The animal traverses shorter and shorter distances before making sharp reverse turns to the left. The crawling phase ends abruptly when the larva extends the foot to the right and the shell is pulled over on the left valve. The foot contracts several times and cement flows out of the byssus gland. The left valve is held down against the cement for several minutes before the foot withdraws. (For review see: Nelson, 1924; Prytherch, 1934; Truitt, 1944; Galtsoff, 1964; Cranfield, 1973.)

Very little is known about the effects of hypoxia and microxia on the success of bivalve larval settlement. While both May (1973) and Abbe (1986) attribute localized oyster settlement failure to incidents of pycnocline tilting, they present no direct evidence. The only laboratory study of the effects of oxygen deprivation on settlement success is that by Wang and Widdows (1991) on the mussel *Mytilus edulis*. They report that moderate hypoxia has little effect on larval settlement. Settlement of mussel larvae is unaffected down to 1.3 mg O_2 l^{-1} (3.16 kPa pO_2, 15% of air saturation at 15°C and 31 ppt) but is reduced at lower oxygen concentrations.

Metamorphosis

Attachment to a hard substrate marks a radical change in the form and habit of the oyster. This change in habit is accompanied by modifications in the internal organization of the animal, termed metamorphosis. During the transformation from larva to juvenile, larval organs disappear, adult organs form, and larval-adult organs develop. Specifically, the pigmented spots "eyespots" disappear, the velum and foot atrophy, the number of gill filaments increases, and the posterior adductor muscle develops. Metamorphosis has more often been studied in *Ostrea edulis* Linné than in *C. virginica* but the process is probably similar in both species. In reviewing several papers on metamorphosis (Stafford, 1913; Cole, 1937; Cole, 1938; Hickman and Gruffydd, 1971; Fioroni, 1982) it is possible to develop a general time line of metamorphic events. Pigmented "eyespots" disappear in 24 to 48 h following cementation. Both the velum and the foot atrophy and disappear by the third day. About this time, the labial palps appear. Gill rudiments, present in the larvae, lengthen into filaments and increase in number as metamorphosis progresses. The left gill is larger than the right. The anterior adductor muscle disappears while the posterior adductor increases in size and moves ventral.

The effects of hypoxia and microxia on the process of bivalve metamorphosis have not been studied. Other

morphogenetic processes, however, such as embryonic development, are affected by oxygen deprivation. Larvae and eggs of the hard clam, *Mercenaria mercenaria*, do not develop normally below oxygen levels of 4.2 and 0.5 mg O_2 l^{-1}, respectively (60% and 7% of air saturation at 25°C and 28-30 ppt) (Morrison, 1971). In a recent paper on larvae of the mussel, *M. edulis*, Wang and Widdows (1991) report arrested and abnormal embryonic development under hypoxic conditions of 0.6 mg O_2 l^{-1} (1.38 kPa pO_2, 7% of air saturation).

Feeding

Both larval and adult bivalves are filter feeders; they gather algal cells, bacteria, and protists from the water column using cilia. In larvae, particles are captured by patterns of fluid flow and shear gradients, rather than by actual contact with the the velar cilia (Gallager, 1988). In *Crassostrea gigas* (Thunberg, 1793) larvae, absolute clearance rates are greatest for veliger larvae and decline as they reach the pediveliger stage (Gerdes, 1983). In adult oysters, clearance rate increases with body size (Gerdes, 1983). The mode of function of lamellibranch gills is not fully understood and is a matter of some debate. (For review see: Barnes, 1987; Griffiths and Griffiths, 1987; Jørgensen, 1990.)

Under optimum conditions, bivalves filter particles at a constant rate. If conditions are not optimal (e.g.,

mechanical disturbance, sedimentation, toxicants, low oxygen), bivalves close or partially close their valves and filtration and feeding declines or stops (Jørgensen, 1990). Low dissolved oxygen conditions of 1.1 mg O_2 l^{-1} (0.8 ml O_2 l^{-1}, 14% of air saturation at 22°C and 17 ppt) inhibit the ingestion of food by adult *C. virginica* (Haven and Bendl, unpublished). In some adult bivalves the relationship between dissolved oxygen and clearance rate appears to be more complicated. In adult *M. edulis*, clearance rates increase as dissolved oxygen decreases from saturation to approximately 4.5 mg O_2 l^{-1} (80 mm Hg, 53% of air saturation at 15°C and 31 ppt) and subsequently decline at lower oxygen concentrations (Bayne, 1971). Similar responses are observed in larvae. Widdows *et al.* (1989) studied the effects of hypoxia and microxia on both the rate of ingestion and on the proportion of *C. virginica* larvae feeding. Prodissoconch, veliconch and pediveliger larvae show a reduction in the proportion of larvae feeding and a decline in ingestion rates when exposed to hypoxia. Prodissoconch and veliconch larvae show a small reduction compared to pediveligers. Prodissoconch and veliconch larvae maintain relatively high ingestion rates (24-40% of normoxic rates) for 24 h under all hypoxic conditions less than 2.2 mg O_2 l^{-1} (< 5.6 kPa pO_2, less than 27% of air saturation) and feeding stops after 10 h in microxia. Pediveliger larvae have ingestion rates of less than 4% of

normoxic rates after 24 h under hypoxic conditions and in microxic conditions feeding ceases after only 4 h of exposure (Widdows *et al.*, 1989). *Mytilus edulis* larvae also show an increase in the sensitivity of feeding activity to oxygen deprivation with body size. Like adult mussels, prodissoconch and veliconch larval mussels exhibit maintained or enhanced feeding down to 1.3 mg O_2 l^{-1} (Wang and Widdows, 1991).

Growth

The portion of dietary intake above that required for maintenance of normal processes is available for growth (Parry, 1983). The ability to grow is influenced by food supply, temperature, and salinity, among other things (Griffiths and Griffiths, 1987). Little is known about the effects of low oxygen conditions on growth. A few authors have examined the effects of hypoxia on larval growth. The larval growth rate of *M. mercenaria* is depressed at oxygen levels below 4.2 mg O_2 l^{-1} (Morrison, 1971). The sensitivity of *M. edulis* larval growth to hypoxia varies with body size. The growth of prodissoconch and veliconch larvae is maintained in oxygen concentrations at or above 1.3 mg O_2 l^{-1} while the growth of later larval stages is depressed in concentrations at or below 2.4 mg O_2 l^{-1} (5.91 kPa pO_2, 29% of air saturation) (Wang and Widdows, 1991).

Metabolism

Metabolism is the oxidation of food materials, ultimately carbon and hydrogen, for the purpose of producing energy (ATP) for biological work. Oxygen is required for complete oxidation but ATP can also be generated in the absence of oxygen. In anaerobic metabolism, carbohydrate (glycogen) is fermented, in a process referred to as glycolysis, which in species other than molluscs, yields about one twelfth as much ATP as is produced in aerobic metabolism. (For review see: Gordon, 1982; Schmidt-Nielsen, 1985; Prosser, 1986.) Molluscs are unique because they have more than one anaerobic pathway. While vertebrates have only one anaerobic pathway leading to lactate as the sole end product, bivalves have three anaerobic pathways leading to a variety of end products other than lactate (Hammen, 1976; de Zwaan and Wijsman, 1976). ATP is produced, not only by glycolysis as in anaerobically metabolizing vertebrates, but also by oxidative phosphorylation which produces more ATP per unit fuel. It is important to note that all anaerobic metabolism produces less ATP per unit of fuel than does aerobic metabolism but that the pathways used by bivalves produce more than that used by vertebrates. Energy demands may also be partially met under anaerobic conditions by depletion of the ATP and phosphagen pools (de Zwaan and Wijsman, 1976). As anaerobic metabolism proceeds, there is a shift in the ratios of the various end products

and in the rates of glycogen fermentation and ATP turnover (de Zwaan and Wijsman, 1976; Widdows, 1987). The process of switching from aerobic to anaerobic metabolic pathways is not well understood. It appears that in many bivalves, anaerobic pathways are in constant operation, to some degree, and increase as oxygen availability decreases (Hammen, 1976; Hammen, 1980; Widdows, 1987). When oxygen is again available, the end products of anaerobic metabolism are oxidized or converted back to storage products, and phosphagen and ATP pools are recharged (Herreid, 1980; Ellington, 1983). These processes require oxygen and result in an oxygen consumption rate, upon return to aerobic conditions, beyond that which is expected under normal aerobic conditions. This increase in oxygen consumption is considered repayment of the oxygen "debt".

Metabolic rate can be measured directly as the rate of heat dissipation, or indirectly as the rate of oxygen consumption or the rate of accumulation of anaerobic end products. Heat is produced as metabolic substrates are oxidized. Thus, total metabolism can be measured directly as the amount of heat dissipating from an animal (Gnaiger, 1983b; Widdows, 1987). Heat dissipation is technically difficult to measure and so indirect methods of measuring metabolism are often employed. Oxygen consumption rate is fairly easy to measure but has limitations. The use of oxygen consumption to estimate total metabolism assumes that

metabolism is totally aerobic (Widdows, 1987). Many bivalves, however, are partially anaerobic even under oxygenated conditions (Hammen, 1979). Ideally, both heat dissipation and oxygen consumption are measured simultaneously. Oxygen consumption is converted to heat dissipation equivalents. The anaerobic component of metabolism is determined by subtracting the aerobic heat dissipation equivalents from the direct measure of total metabolism (Gnaiger, 1983b; Pamatmat, 1983; Widdows, 1987).

Numerous authors have examined the effects of declining oxygen concentration on oxygen consumption and animals have been categorized by their respiratory responses as either oxyregulators or oxyconformers. In oxyconformers, oxygen consumption varies in direct proportion to the environmental oxygen concentration, while in oxyregulators, oxygen consumption is relatively constant down to some critical oxygen concentration at which the animal becomes an oxyconformer (For review see: Prosser, 1986; Griffiths & Griffiths, 1987; Herreid, 1980.) Oxyconformity and oxyregulation represent two ends of a broad spectrum of responses (Mangum and van Winkle, 1973). Oxyregulators compensate for declining oxygen concentration by increasing ventilation of respiratory surfaces and/or oxygen extraction efficiencies (Herreid, 1980; Widdows, 1987; Griffiths and Griffiths, 1987). No animal is a perfect oxyregulator over the entire range of oxygen concentrations. At some critical

oxygen concentration, oxyregulators become oxyconformers. This critical concentration varies with individual, temperature, salinity, and previous exposure to hypoxia (Herreid, 1980; Shumway, 1982; Griffiths and Griffiths, 1987).

Total metabolism also varies with oxygen concentration. Herreid (1980) has identified three types of responses: homeometabolic, poikilometabolic, and heterometabolic responses. The homeometabolic response refers to a maintenance of total energy expenditure despite a decrease in oxygen availability. In this case, anaerobic metabolic pathways compensate for a decrease in aerobic metabolism. At the other extreme, total metabolism decreases directly with the decline of oxygen and no anaerobic metabolism occurs under hypoxic conditions. This response is termed poikilometabolic. Heterometabolism is the intermediate response. In this case, total metabolism decreases but some anaerobic metabolism occurs. Except for obligate anaerobes, most animals are either poikilometabolic or heterometabolic; metabolism under hypoxic conditions falls short of total metabolism under normoxic conditions (Herreid, 1980). Response to microxia is usually characterized by extremely low levels of metabolism (Gnaiger, 1983b; Widdows, 1987).

Crassostrea virginica adults are able to oxyregulate over a wide range of oxygen concentrations but switch to oxyconformity at about 1.5 mg O_2 l^{-1} (30 mm Hg, 20% of air

saturation at 20°C and 28 ppt) depending on temperature and salinity (Shumway, 1982; Shumway and Koehn, 1982). Heat dissipation rates, however, show that even during maximum aerobic metabolism, some anaerobic metabolism occurs (Hammen, 1979; Hammen, 1980). Stickle *et al.* (1989) report microxic metabolic rates of 75% of the aerobic rates. These animals were starved, however, and so aerobic rates were probably low.

Widdows *et al.* (1989) studied the responses of *C. virginica* larvae and juveniles (16 mm height) to declining oxygen and microxia. Both larvae and juveniles are good oxyregulators. Prodissoconch and veliconch larvae maintain heat dissipation and oxygen consumption rates down to 0.8 mg O_2 l^{-1} (2 kPa pO_2, 9.5% of air saturation), and pediveligers and juveniles maintain down to 3.1 mg O_2 l^{-1}. Metabolism is mainly aerobic above these critical oxygen concentrations. Below the critical oxygen concentrations, metabolic rates are dependent on the external oxygen concentration. At oxygen concentrations equal to or below 0.3 mg O_2 l^{-1} (0.67 kPa pO_2, 3% of air saturation) and 0.8 mg O_2 l^{-1}, larval stages and juveniles, respectively, have a large anaerobic component to their total metabolism. Under microxic conditions prodissoconch and veliconch larvae maintain heat dissipation rates at 34% of the normoxic rate while pediveligers and juveniles maintain heat dissipation at only 3% of the normoxic rate. As microxic conditions

continue, these values decrease still further (Widdows *et al.*, 1989).

Tolerance of low dissolved oxygen

Bivalves respond to hypoxia and microxia by closing their valves and metabolizing anaerobically (Akberali and Trueman, 1985). Tolerance to microxia appears to be related to the ability to reduce rates of metabolism and conserve energy. Tolerance of microxia is represented by median mortality time (time to 50% mortality) and is dependent on body size and temperature. Widdows *et al.* (1989) report median mortality times of 11, 18, and 51 h for consecutive *C. virginica* larval stages and pediveligers. Juveniles (16 mm height) have a median mortality time of 150 h. Adult oysters have median mortality times of 5 d, 19 d, and greater than 28 d at temperatures of 30°C, 20°C, and 10°C, respectively (Stickle *et al.*, 1989). At 5°C adult oysters can survive for up to 5 w (Dunnington, 1968).

Methods of Investigation

Each of the chapters has been written as a publishable unit, each with a materials and methods section. The following is an explanation and justification of the methods used.

Chapter 2 examines the effects of hypoxia and microxia on settlement of oyster (*Crassostrea virginica*) pediveliger larvae and on the growth and survival of post-settlement oysters. A flow-through system was employed as the best method of delivering seawater of constant oxygen concentration to several experimental chambers. Microxic and hypoxic tolerance was determined by median mortality times. Shell height (umbo to distal shell margin) was measured as an index of post-settlement growth. The inhibition of growth which occurs under hypoxia is adequately quantified by shell length because hypoxia does not change the relationship between weight and shell length (Wang and Widdows, 1991).

In Chapter 3 a scheme of metamorphic phases, that are easily identifiable in live animals, is developed and used in examining the effects of hypoxia and microxia on metamorphosis. Observations and photographs taken at intervals during the metamorphic process were used to develop a scheme of easily identifiable phases. These

phases were used in examining the metamorphic process during and following continuous and short term exposures to hypoxia and microxia.

The objectives of Chapter 4 were to determine when or if feeding is interrupted during the metamorphic process, and to examine the effects of hypoxia and microxia on the rate of particle clearance by post-settlement oysters. Fluorescent microspheres were used to observe when or if ingestion is interrupted during metamorphosis. The effects of hypoxia and microxia on clearance rates of post-settlement oysters were quantified using a Coulter Counter. Fluorescent microspheres were also used to determine the proportion of post-settlement oysters feeding following 24 h exposures to hypoxia and microxia.

The purpose of Chapter 5 was to determine the heat dissipation and oxygen uptake rates of metamorphosing oysters and to examine the effects of hypoxia and microxia on those rates. Ideally, both oxygen consumption and heat dissipation rates would have been determined simultaneously using a Thermal Activity Monitor perfusion system and Twin-flow Respirometer at the University of Maine.

The LKB Thermal Activity Monitor consists of a thermostated water bath, up to four calorimetric channels, and electronics. Each twin calorimetric channel consists of two measuring cups and Peltier elements. Samples are held in 5 ml stainless steel ampoules. One measuring cup

contains a sample ampoule and the other a reference ampoule of identical mass. The resulting signal is the difference in heat flow between the two ampoules. Electronic calibration is accomplished by passing known voltages through precision resistors (Suurkuusk and Wadsö, 1982).

Unfortunately, I was only able to obtain heat dissipation rates during the limited time available with the instrument. Oxygen consumption rates were, therefore, measured using polarographic oxygen sensors held in microrespiration cells. Comparisons of total metabolic rates and oxygen consumption are limited because they were not obtained concurrently.

The constant experimental conditions created in these experiments do not duplicate natural systems. The results of these experiments are, however, indicative of the effects of low dissolved oxygen and may be used to form the basis of more complex experimental designs.

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Chapter 2

EFFECTS OF HYPOXIA AND MICROXIA ON LARVAL SETTLEMENT, POST-SETTLEMENT GROWTH, AND POST-SETTLEMENT SURVIVAL OF THE OYSTER, *CRASSOSTREA VIRGINICA*.

Abstract

The effects of hypoxia (1.5 mg O_2 l^{-1}, 20% of air saturation) and microxia (< 0.07 mg O_2 l^{-1}, <1% of air saturation) on oyster (*Crassostrea virginica*) larval settlement, juvenile growth, and juvenile survival were studied. I tested the null hypotheses that larval settlement and mortality were equal in normoxic, hypoxic, and microxic treatments and that there were no differences in the slope or elevations of growth regression lines. Settlement was reduced significantly ($P < 0.05$) in hypoxic treatments, as compared to normoxic treatments (7.3 mg O_2 l^{-1}, 100% of air saturation), and almost no settlement took place in microxic treatments. In the first 144 h after settlement, post-settlement oysters in hypoxic treatments grew at a rate one third as much as those in normoxic treatments, while post-settlement oysters in microxic treatments did not grow at all. Median mortality times of post-settlement oysters in hypoxic and microxic treatments were 131 h and 84 h, respectively. I conclude that hypoxic and microxic waters have potentially detrimental effects on oyster settlement and recruitment.

Introduction

Many bays throughout the range of the Eastern Oyster, *Crassostrea virginica* (Gmelin, 1791), (*eg.* Long Island Sound, Chesapeake Bay, Pamlico Sound) exhibit 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 microxic water from deeper areas (May, 1973; Sanford, 1990). The pycnocline remains tilted for from several hours to 2 or 3 d (Malone *et al.*, 1986; Sanford *et al.*, 1987). These events often coincide with the timing of settlement and recruitment of the oyster, *C. virginica*. 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 microxia increases with developmental stage and body size. Larval stages and juvenile oysters (16 mm height) survive microxia 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 oyster larvae in the process of settlement or of post-settlement oysters to hypoxia and microxia. These stages are pivotal to subsequent recruitment into the population. The objectives of my study, therefore, were to examine the effects of low oxygen on settlement of oyster pediveliger larvae and on the growth and survival of post-settlement oysters. I tested the null hypotheses that larval settlement and mortality were equal in normoxic, hypoxic, and microxic treatments. I also tested the null hypothesis that there were no differences in the slopes or elevations of growth regression lines.

Materials and Methods

Experimental apparatus

All experiments were performed at 25°C and 21 ppt salinity. Temperature was maintained by control of the 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 microxia, respectively. Although carbon dioxide was not in the bottled gas of the latter two treatments, pH did not differ significantly (ANOVA, P = 0.287) among the three treatments.

Flow-through chambers were constructed to hold larval and post-settlement 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. Tygon tubing was used in the peristaltic pump and stainless steel tubing (1 mm bore) was used throughout the rest of the system. Flow rate through the chambers was about 233 ml h^{-1}, and water residence time in the system was one h or less. The flasks of seawater and algae were replaced every 12 h with identical flasks which 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 (MC 100) 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 microxic treatments were consistently maintained at 85-100%, 15-22% and 0-1% of 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, VA. 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 (2 x 1.5 cm) was placed in each chamber with the rough side up. Fifty larvae (298 μm shell height, umbo to distal shell margin) 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 without replacement. 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 microxic 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's multiple comparison test was performed to determine where differences in treatment means existed (Zar, 1984). Means and standard deviations were back transformed for reporting in Figure 2.

Post-settlement growth and survival experiments

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 without replacement. Twenty-five randomly selected live post-settlement 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 post-settlement oysters among 50 randomly selected post-settlement oysters from each chamber. The data from the two chambers were pooled as one replicate for that exposure/post-settlement time. The entire post-settlement growth and survival experiment was repeated four times resulting in four replicates of normoxic treatments, and three replicates each of hypoxic and microxic treatments.

Growth data were $\log_{10}$ 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 post-settlement 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 differences between treatment means (Zar, 1984). Means and standard deviations were back transformed for reporting in Figure 4.

Results

Larval settlement

In normoxic treatments at 24 h, mean settlement of oyster (*Crassostrea virginica*) larvae was 38% (Fig. 2). The percentage of settled larvae increased 10-20% d^{-1}, and was 79% at 96 h. In the hypoxic treatments, settlement was 18% at 24 h and 38% at 48 h. After 48 h, no further settlement occurred in hypoxic treatments. In microxic treatments, settlement was 4% at 24 h, with no subsequent settlement. At 24 h microxic and normoxic treatment means were significantly different (Tukey's test, $P = 0.022$), and at 48 h the microxic treatment mean was significantly different (Tukey's test, $P \leq 0.008$) from both the hypoxic and normoxic treatment means. At 72 and 96 h all three treatment means were significantly different (Tukey's test, $P \leq 0.044$) from each other.

Post-settlement growth

Regressions of $\log_{10}$ transformed post-settlement growth data from normoxic and hypoxic treatments were linear and significant (ANOVA, $P \leq 0.05$). The regression coefficients of the normoxic and hypoxic treatments were not significantly different (Student's t test, $P > 0.50$); however, the regression elevations were significantly

different (Student's t test, $P < 0.001$) from each other (Fig. 3). Post-settlement oysters in the normoxic treatments grew over 255 μm of new shell in 144 h, nearly doubling in length. Post-settlement oysters in hypoxic treatments grew 77 μm of new shell in 144 h, approximately one third as much as those in normoxic treatments. Post-settlement oysters in microxic treatments did not increase in shell height.

Post-settlement survival

Post-settlement survival was not significantly different (ANOVA, $P \geq 0.055$) in the three treatments for the first 72 h (Fig. 4). At 96 h and 120 h the microxic treatment mean was significantly different (ANOVA, $P = 0.020$, $P = 0.001$, respectively) from both hypoxic and normoxic treatment means. All three treatment means were significantly different (ANOVA, $P = 0.001$) from each other at 144 h. Post-settlement oysters in the microxic treatments had a median mortality time (time to 50% mortality) of 84 h. Mortality of post-settlement oysters in microxic treatments was 100% by 144 h. Post-settlement oysters in the hypoxic treatments had a median mortality time of 131 h. Normoxic treatments, in contrast, had a mean of 13% mortality at 144 h.

Discussion

Under hypoxic and microxic conditions, oyster (*Crassostrea virginica*) pediveliger larvae reduce energetically expensive activities, thereby reducing total metabolism and oxygen requirements (Widdows *et al.*, 1989). The results of my study indicate that settlement is another costly activity that oyster pediveliger larvae are unable to complete when in oxygen limiting environments.

In a recent paper on the effects of hypoxia and microxia on the larvae of *Mytilus edulis* Linné, 1758, 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 2 d in treatments of 8.2 mg O_2 l^{-1} (20.0 kPa pO_2, 98% of air saturation at 15°C and 31 ppt), 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 reduced by oxygen concentrations of 1.5 mg O_2 l^{-1} or less. The

estimated oxygen concentration at which settlement after 2 d 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 ppt) 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 study) 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, under hypoxic conditions pediveliger larvae reduce energetically expensive activities, 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 under hypoxic conditions (Wang and Widdows, 1991). The reduction of post-settlement growth in hypoxic treatments and complete lack of growth in microxic treatments observed in this study may have resulted from a cessation of feeding.

In my study, post-settlement oysters had a median mortality time of 84 h in microxia. This indicates that, like oyster larvae and adults, recently settled post-settlement oysters are capable of anaerobic metabolism. Widdows *et al.* (1989) report median mortality times in microxia 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 post-settlement oysters are consistent with the trend of increasing microxic 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 microxic conditions (Widdows *et al.*, 1989). The degree of heat dissipation reduction by post-settlement oysters in microxia 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 more clearly understand the effects of microxia and hypoxia on settling pediveliger larvae and post-settlement oysters. The present study does demonstrate that hypoxic and microxic conditions have detrimental effects on larval settlement, post-settlement growth, and post-settlement survival. Oyster distribution may be influenced by microxia and hypoxia, especially in those areas that experience prolonged (longer than 48 to 72 h) or severe (microxic) pycnocline tilt events. Pycnocline tilt events may control recruitment into the adult population directly, due to larval settlement failure and post-settlement mortality, and indirectly, due to a reduction in the growth rate of post-settlement oysters.

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

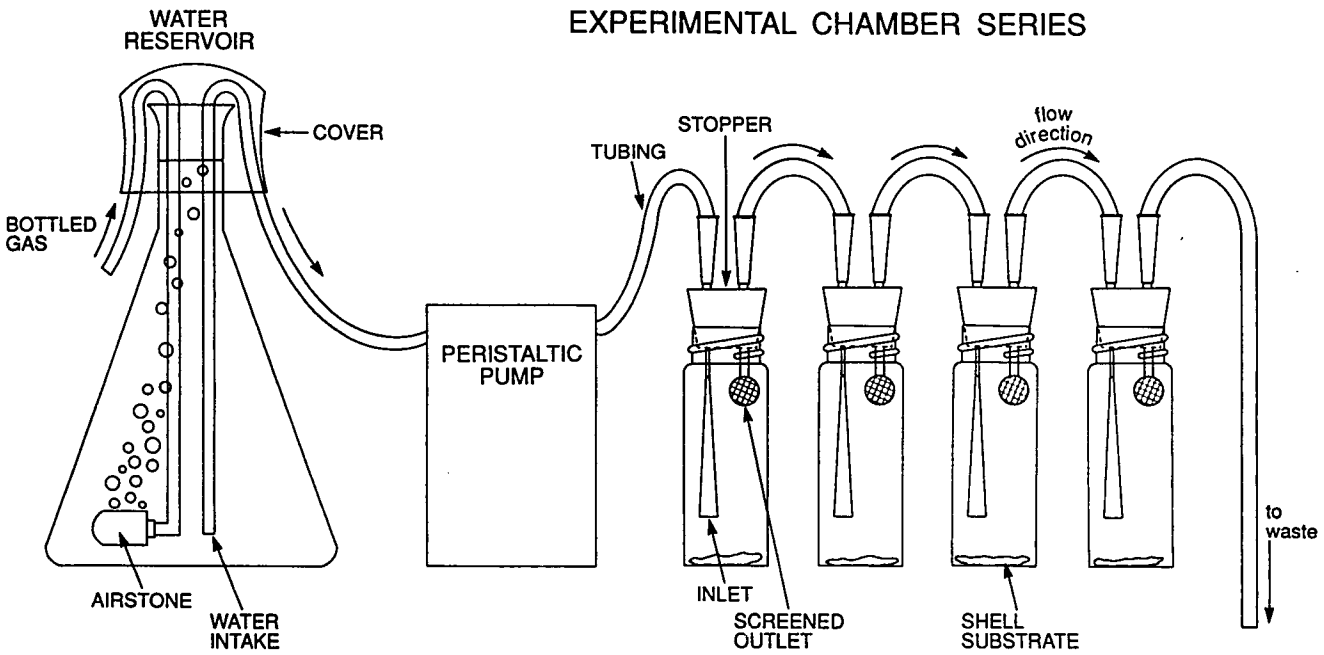

EXPERIMENTAL CHAMBER SERIES
WATER RESERVOIR
COVER
BOTTLED GAS
AIRSTONE
WATER INTAKE
PERISTALTIC PUMP
TUBING
STOPPER
flow direction
INLET
SCREENED OUTLET
SHELL SUBSTRATE
to waste

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

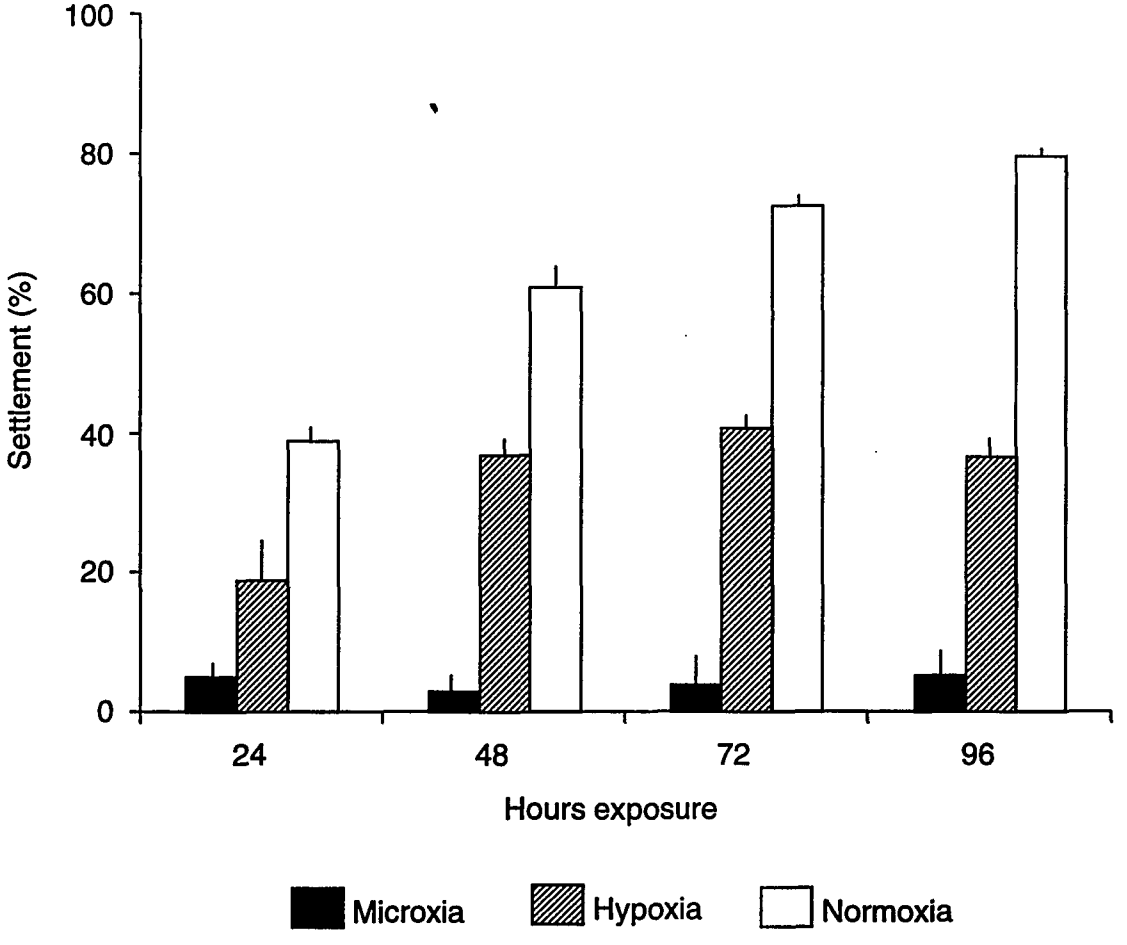

100
80
60
40
20
0
Settlement (%)
24
48
72
96
Hours exposure
Microxia
Hypoxia
Normoxia

Figure 3. *Crassostrea virginica*. Log_{10} of growth of post-settlement oysters (initial shell height, umbo to distal shell margin, 290 μm) in normoxic (7.3 mg O_2 l^{-1}), hypoxia (1.5 mg O_2 l^{-1}), and microxic (< 0.07 mg O_2 l^{-1}) treatments in relation to h post-settlement. (Means ± SD. Normoxia n = 175 for each mean marker. Hypoxia n = 125 for each mean marker.)

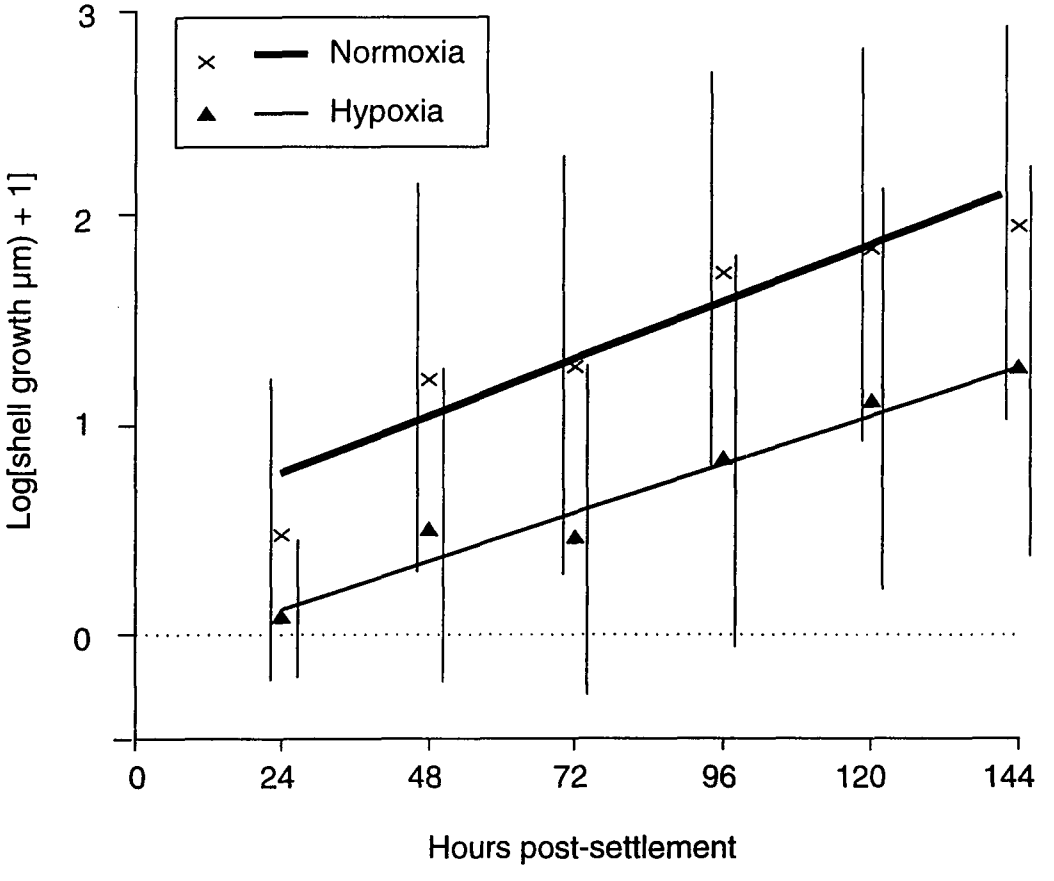
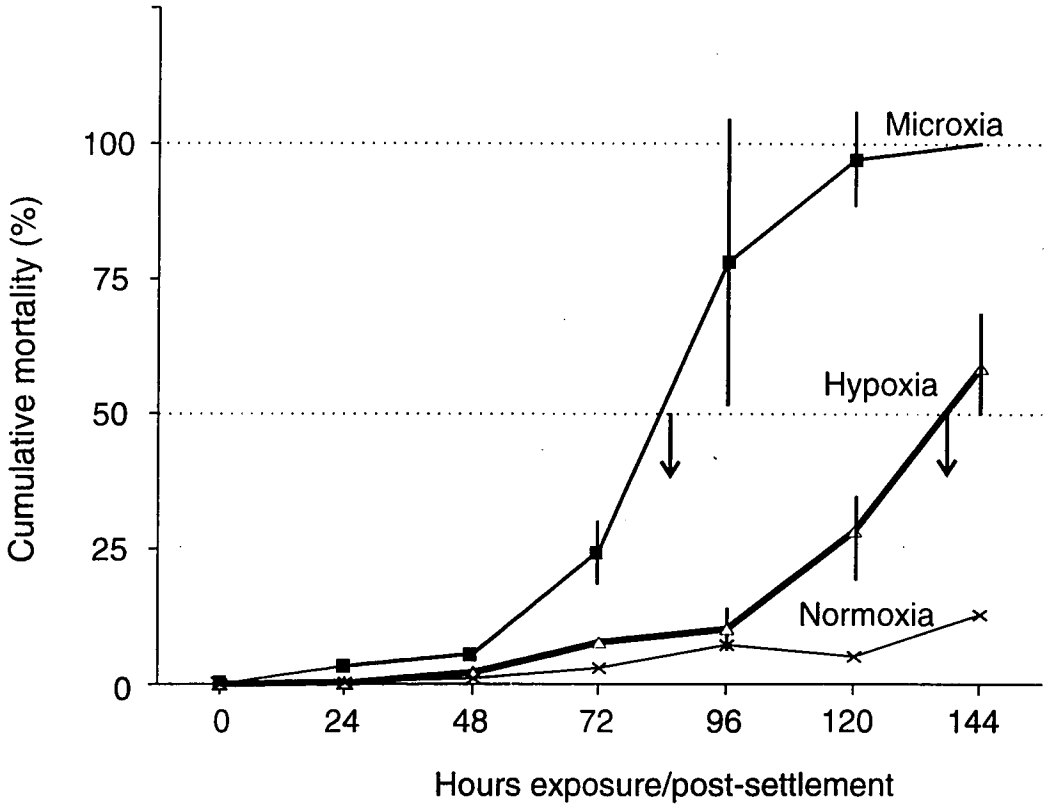

Normoxia
Hypoxia
Log[shell growth µm) + 1]
3
2
1
0
0
24
48
72
96
120
144
Hours post-settlement

Figure 4. *Crassostrea virginica*. Relation between cumulative mortality of post-settlement oysters and duration of normoxic (7.3 mg O_2 l^{-1}), hypoxic (1.5 mg $O_2 l^{-1}$), and microxic (< 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. Microxia n = 3.)


Microxia
Hypoxia
Normoxia
Cumulative mortality (%)
100
75
50
25
0
0
24
48
72
96
120
144
Hours exposure/post-settlement

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Chapter 3

DESCRIPTION OF METAMORPHIC PHASES IN THE OYSTER *CRASSOSTREA VIRGINICA* AND EFFECTS OF HYPOXIA ON METAMORPHOSIS

Abstract

Four phases of metamorphosis in the eastern oyster, *Crassostrea virginica*, were characterized: **Settlers** have attached to the substrate but retain larval characteristics; metamorphosis and degeneration of the velum has begun in **prodissoconch postlarvae**; in **dissoconch postlarvae**, shell growth beyond the prodissoconch has begun but the foot persists; and, **juveniles** have lost all larval organs and metamorphosis is complete. These phases were used in examining the metamorphic process during and following continuous and short term exposures to hypoxia (1.5 mg O_2 l^{-1}, 20% of air saturation) and microxia (< 0.07 mg O_2 l^{-1}, < 1% of air saturation). I tested the following null hypotheses: A) No effect of continuous oxygen treatment on the number of oysters completing metamorphosis, B) No effect of oxygen treatment, length of exposure, or interaction of treatment and exposure time, on the number of oysters completing metamorphosis, and C) No differences in the slopes or elevations of growth regression lines of post-settlement oysters following exposure to oxygen treatments for different periods. I observed no abnormal development in the oysters, but development was delayed 3 or more days following 3 d exposures to hypoxia, and 2 and 3 d exposures to microxia. Under continuous exposure to microxia, oysters

did not develop to the dissoconch postlarva or juvenile phases. Approximately 50% of the control oysters died within the 14 d period following settlement. Mortality was virtually confined to the settler and prodissoconch postlarva phases. Short-term exposures to hypoxia (1 to 3 d) and microxia (1 d) had little effect on the median mortality time or final total mortality, compared to controls. Microxic treatments longer than 1 d increased mortality and oysters continuously exposed to microxia had a median mortality time of 87 h. Short term exposures to low oxygen did not have permanent effects on post-settlement growth rates. Animals exposed to microxic treatments, however, appeared to have slower growth rates during the exposure period. I conclude that low oxygen conditions, in particular those that are microxic and last longer than 24 h, have detrimental effects on the development, growth, and survival of post-settlement oysters.

Introduction

Some coastal embayments and estuaries, such as Long Island Sound, Chesapeake Bay, and Pamlico Sound exhibit oxygen depletion of deep waters as the result of density stratification during the summer months (Taft *et al.*, 1980; Officer *et al.*, 1984). Wind forcing may tilt the pycnocline, thereby moving hypoxic or microxic deep water into shallow areas where oyster reefs occur (May, 1973; Malone *et al.*, 1986). Irrigation of shallow areas may continue for several hours to 2 or 3 d (Sanford *et al.*, 1990). Oxygen depletion of deep waters occurs during warm summer months. Therefore, hypoxic intrusion onto the shallow flanks of these bays and sounds often coincide with the period of settlement and subsequent metamorphosis of the oyster *Crassostrea virginica* (Gmelin, 1791).

Many morphogenic processes, such as embryonic development and growth of larvae, are affected negatively by oxygen deprivation. Exposure to hypoxia causes delayed and abnormal embryonic development and reduced larval growth rates in both the clam *Mercenaria mercenaria* and the mussel *Mytilus edulis* (Morrison, 1971; Wang and Widdows, 1991). Metamorphosis is a morphogenic process in which larval organs disappear and permanent adult organs reorganize and

develop (Fioroni, 1982); yet, it is not known what effects low oxygen conditions have on bivalve metamorphosis.

Effects of low oxygen exposure on metamorphosis must necessarily be evaluated against normal metamorphic processes. Although several authors have observed the morphology of post-settlement oysters (Jackson, 1888; Stafford, 1913; Cole, 1937; Cole, 1938; Hickman and Guffydd, 1971), no description of metamorphosing *C. virginica* identifies specific morphogenic phases in live oysters.

The objectives of my study included first developing a scheme of metamorphic phases that are easily identifiable in live *C. virginica*. Then, using this scheme, I examined the process of metamorphosis during continuous exposure to low oxygen, as well as during recovery periods following exposure. I tested the following null hypotheses: A) There was no effect of oxygen treatment on the number of oysters completing metamorphosis, B) There was no effect of oxygen treatment, length of exposure, or interaction of treatment and exposure time, on the number of oysters completing metamorphosis, and C) There were no differences in the slopes or elevations of growth regression lines of post-settlement oysters following exposure to oxygen treatments for different periods.

Materials and Methods

Metamorphic phases

Pediveliger larvae of the eastern oyster, *Crassostrea virginica* were obtained from the Virginia Institute of Marine Science hatchery at Gloucester Point, VA. Frosted Mylar acetate settlement substrates were held in seawater for at least 2 d prior to each experiment to develop a settlement-inducing bacterial coating (Fitt *et al.*, 1990). Larvae (299 ± 18 μm mean shell height, umbo to distal shell margin, S.D., n = 10) were exposed to a conditioned settlement substrate unit for 1 h. Larvae that had not settled and attached within that time were rinsed from the substrate unit. Ten newly-attached larvae were labeled by marking their location on the substrate unit with pencil. The substrate unit and attached oysters were maintained in a glass dish in filtered (0.45 μm) seawater of 21 ppt at an ambient temperature of 25°C . Water was changed daily and oysters were fed *Isochrysis galbana* at a concentration of 20,000 cells ml^{-1}.

The internal morphology of labeled oysters was examined and photographed using an Olympus SAH zoom stereo microscope (maximum magnification 64X) and illumination base equipped with a phototube and a Contax (137 MA Quartz) 35 mm camera. Examinations were made at 3 to 24 h intervals, beginning at

3 h post-settlement. The photographs and notes were used to develop a scheme of metamorphic phases based on the condition of the velum, foot, gills, labial palps, and shells. Figures were made with a microscope drawing attachment.

Continuous low oxygen experiments

Treatment flasks of seawater, containing algal food (*Isochrysis galbana*) at a concentration of 20,000 cells ml^{-1}, were continuously bubbled with either 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 were referred to as normoxic, hypoxic, and microxic, respectively. Oxygen concentrations were measured twice a day with a Strathkelvin Instruments (SI) oxygen sensor (1302) coupled to an SI oxygen meter (781) and chart recorder. The oxygen sensor was calibrated with air-saturated water (100% of air saturation) and a zero oxygen solution of sodium borate and crystalline sodium sulfite. Normoxic, hypoxic, and microxic treatments were consistently maintained at 90-100%, 17-24%, and 0-1% of air saturation, respectively.

The metamorphic phases of oysters were determined after 24 to 120 h of continuous exposure to the three oxygen

treatments, as follows. Pediveliger oyster larvae were exposed to a conditioned substrate for 3 h. The substrate was cut into 1 X 2 cm pieces so that each substrate unit had at least 10 newly-attached oysters. Five substrate units were suspended in each of three 250 ml flasks (one flask for each oxygen treatment). The flasks were covered with inverted beakers and continuously bubbled with gases. One substrate unit was removed from each treatment every 24 h without replacement so that different individuals were examined each day. The metamorphic phases to which ten oysters had developed were determined immediately. Remaining substrate units were transferred daily to identical flasks of seawater and algae that had been bubbled with the appropriate gases for at least 1 h. The continuous exposure experiment lasted 120 h and was repeated three times (three replicates) with different cohorts of larvae.

For each oxygen treatment-exposure time replicate counts of individuals in the different metamorphic phases were converted to percentages of the total number of individuals observed. The percentages were arc sine-transformed. A one-way analysis of variance was performed to test the null hypothesis that there was no effect of oxygen treatment on the proportion of oysters completing metamorphosis to the juvenile phase by the end of 120 h of continuous exposure. Tukey's multiple comparison test was used to identify differences between specific oxygen

treatments. Statistical analyses were conducted using Minitab software. Data from the continuous experiments are reported as the back-transformed means and standard deviations of the three replicates using h as the time scale.

Recovery experiments

Metamorphic phases and growth of individual oysters were examined during recovery following 1, 2, or 3 d of exposure to the three oxygen treatments. On Day 0, pediveliger larvae were allowed to settle onto conditioned substrate that was then cut into 2 X 7 cm substrate units. Fifteen newly-attached oysters on each substrate unit were labeled with pencil on the adjacent substrate. Substrate units were placed in separate covered beakers (250 ml) of seawater and algae through which gases were continuously bubbled. There were a total of 9 beakers; one per oxygen treatment-exposure time combination.

On Day 1, one substrate unit from each oxygen treatment was examined. Substrate units were kept in glass dishes full of water during examination. The metamorphic phase to which each of the 15 labeled oysters had developed was determined and the oysters were measured along the dorsal-ventral axis (shell height, umbo to distal shell margin) with a compound microscope fitted with an ocular micrometer. Following examination, the substrate units were returned to

the beakers and subsequently maintained at normoxia for the remainder of the experiment.

On Day 2 another substrate unit from each oxygen treatment was examined. The oysters were observed and measured as described above. These substrate units were then maintained at normoxia for the rest of the experiment. Oysters that had been examined on Day 1 were again measured and the metamorphic phases determined.

On Day 3, the final three unexamined substrate units, one from each oxygen treatment, were examined and placed in normoxic water, as above. Oysters examined on Days 1 and 2 were again re-examined.

Once substrate units had been placed in normoxia, following 1, 2, or 3 d of exposure to an oxygen treatment, the labeled oysters were examined daily until Day 8; after that they were examined every other day. Substrate units in normoxic treatments were treated the same as substrate units in other treatments so that they were not examined until Day 1, 2, or 3, to serve as controls. Each day, during both exposure and recovery periods, substrate units were transferred to fresh beakers of seawater and algae that had been bubbled with the appropriate gases for at least 1 h. The recovery experiment lasted 14 d and was repeated three times with different cohorts of larvae.

For each oxygen treatment-exposure time replicate, counts of individuals in the different metamorphic phases

were converted to percentages of the total number of individuals observed. The percentages were arc sine-transformed. A two-way analysis of variance was performed to test the null hypotheses that there were no effects of oxygen treatment, length of exposure, or interaction, on the proportion of oysters completing metamorphosis to the juvenile phase by the end of 14 d. If a null hypothesis was rejected, Tukey's multiple comparison test was used to identify differences between specific oxygen treatments and lengths of exposure. Data from the recovery experiments are reported as the back-transformed means and standard deviations of the three replicates using d as the time scale.

Growth data were $\log_{10}$-transformed and analyses of variance were used to test the significance of linear regressions. Analysis of covariance was performed to test the null hypotheses that there were no differences in slope or elevation between the nine linear regression lines. If a null hypothesis was rejected, a Tukey's multiple comparison test was used to determine which linear regression lines were different from each other. Statistical analyses were conducted using Minitab software.

Results

Metamorphic phases

Four distinct phases of metamorphosis were defined (Fig. 5).

Settlers. Settlers are oysters that have attached but otherwise retain characteristics of the pediveliger larvae (Fig. 5A). The distinguishing feature of the settler phase is the presence of an intact and active velum and foot. In some larvae the velum is pigmented. The velum and/or foot sometimes protrudes from between the valves and the velar cilia continue to beat in a coordinated manner. As in the pediveliger, the eyespots are prominent. The rudiment of the left gill, which was also present in the pediveliger, is visible. There is no shell growth beyond the prodissoconch. The shell height of settlers was the same as that of the unattached larvae (299 ± 18 μm, S.D., n = 10).

Prodissoconch postlarvae. Metamorphosis has begun and the organs have started an anterior-dorsal rotation (counterclockwise if viewed with the right valve up, Fig. 5B). The distinguishing feature of prodissoconch postlarvae is the degeneration of the velum, leaving a larger visceral cavity. If the velum was pigmented in larval and settler phases, the pigment is now concentrated in the area of the velar remnant. Velar cilia sometimes beat in an irregular

and uncoordinated manner. The foot is present and the right eyespot is still visible. The gill filaments are longer than in the previous phase. The posterior adductor muscle is visible. There is no shell growth beyond the prodissoconch but the mantle sometimes protrudes beyond the prodissoconch. Prodissoconch postlarvae, that later went on to metamorphose, first appeared an average of 18 h post-settlement.

Dissoconch postlarvae. Metamorphosis and rotation of the organs continues in this phase (Fig. 5C). The distinguishing characteristics of dissoconch postlarvae are shell growth beyond the margin of the prodissoconch and the persistence of the foot. The velum is completely gone. The foot or a foot remnant has rotated along with the rest of the body and is mid-ventral, located between the gills at their proximate end. The eyespot has submerged in the tissue, and broken up into a thin line of pigment located more dorsally than the eyespot; I call this line the *eyestreak*. Both the right and left gills are visible but the right is smaller than the left. The gill filaments of the left gill are longer and new filaments have been added at the distal end. The outer and inner labial palps are visible as active lobes. If the velum is pigmented, the inner palps are also pigmented. New shell has grown in a thin line at the ventral edge of the prodissoconch.

Dissoconch postlarvae first appeared an average of 29 h post-settlement.

Juveniles. In juveniles, all larval organs are lost and metamorphosis is complete (Fig. 5D). The eyestreak is usually gone but sometimes persists for several days. Both the descending and ascending limbs of the inner demibranch of the left gill are visible. (The outer demibranchs develop later.) The right inner demibranch is still smaller than the left one. The mantle is fused at the distal end of the left gill. The outer palps form a hood over the mouth and around the anterior end of the gills. The adductor muscle extends past the edge of the prodissoconch with the intestine passing around it posteriorly. The dissoconch extends in wings on either side of the hinge. Both valves are approximately the same size and shape and the whole of the left valve is still attached. Juveniles appeared on average 48 h post-settlement with a mean shell height of 410 ± 40 μm (S.D., n = 20).

Continuous exposure experiment

Although the number of settlers decreased more slowly in the hypoxic treatments than in the normoxic treatments, the proportion of settlers remaining alive at 120 h was approximately 30% in both treatments (Fig. 6). Prodissoconch postlarvae were always observed in low numbers. In normoxic treatments the highest proportion

(40%) of dissoconch postlarvae was observed at 48 h; after that the proportions declined steadily as they became juveniles. The highest proportion of dissoconch postlarvae observed in hypoxic treatments was 22% at 96 h. In microxic treatments, only 1% of the oysters reached the dissoconch postlarva phase and they died within 24 h.

Major portions of the final juvenile counts in the normoxic and hypoxic treatments appeared by 48 to 72 h. Although the difference between proportions of juveniles at 120 h in normoxic and hypoxic treatments was large (51% and 18%, respectively), they were not significantly different (Tukey's test, $P = 0.327$) from each other. They were, however, both significantly different ($P = 0.024$) from the microxic treatments in which no oysters reached the juvenile phase.

Mortality of all metamorphic phases was approximately 8% in both normoxic and hypoxic treatments at 120 h, although variation was higher in the hypoxic treatments. Oysters in the microxic treatments had a median mortality time (time to 50% mortality) of 87 h and mortality was 100% by 120 h. Nearly all oysters in microxic treatments died as settlers, without beginning metamorphosis.

Exposure and recovery experiment

The number of settlers declined fairly steadily in all treatments and the proportions of settlers remaining on Day

14 was between 3% and 13%. Prodissoconch postlarvae were always observed in low numbers and some were still present at the end of the experiment. Dissoconch postlarvae were observed in proportions of less than 21%.

The final proportions of juveniles were between 6% and 30%. Large portions of the final juvenile counts appeared by Day 3, the exceptions being 3 d exposures to hypoxia, in which the number of juveniles doubled between 6 and 10 d post-settlement, and 2 and 3 d exposures to microxia, in which juveniles appeared at 6 to 14 d post-settlement. No significant (ANOVA, $P = 0.191$) effect of exposure time on the proportion of juveniles at 14 d post-settlement could be detected. In the microxic treatments there was a trend of fewer juveniles when exposure time was longer, although exposure time was not statistically significant. There was no interaction of exposure time and oxygen treatment. There was, however, a significant difference (Tukey's test, $P < 0.001$) between the proportions of juveniles on Day 14 in the normoxic and microxic treatments but none could be detected between hypoxic and normoxic treatments (Tukey's test, $P = 0.056$). Variation between replicate experiments was large and greatest in the microxic treatments.

Median mortality times were greater than 10.4 d except for the 2 and 3 d exposures to microxic treatments which had median mortality times of 3.6 d and less than 3 d, respectively (Fig. 7). Mean total mortality by Day 14 was

between 46 and 61% except in treatments exposed to 2 and 3 d of microxia; these treatments had final mean mortalities of 79% and 88%, respectively. Virtually all oysters that died did so as settlers or prodissoconch postlarvae; only one dissoconch postlarva was observed to die, and no juveniles died.

Linear regressions of $\log_{10}$-transformed growth of dissoconch postlarvae and juveniles were significantly different from zero (ANOVA, $P < 0.001$) (Fig. 8). The slopes of the nine oxygen treatment/exposure time combinations were not significantly different (ANOVA, $P = 0.215$) from each other. There were, however, significant differences (ANOVA, $P < 0.0005$) in the elevations of the lines. The elevation of the growth regression of oysters that experienced 2 d exposures to microxia was significantly lower (Tukey's test, $P = 0.036$) than that of the normoxic control. Oysters that experienced 3 d exposures to microxia had a regression elevation that was significantly lower (Tukey's test, $P \leq 0.036$) than that of both the normoxic control and that of oysters exposed for 3 d to hypoxia. Within oxygen treatments, there were no significant differences (Tukey's test, $P \geq 0.435$) in regression elevation between exposure times. The microxic treatments, and the 2 and 3 d exposures to hypoxic treatments had a number of dissoconch postlarvae that appeared late and/or remained small throughout the experiments.

Discussion

Previous descriptions of the morphology of metamorphosing bivalves have been based on specific organs. This method is inadequate for identifying phases of metamorphosis in live oysters because it is difficult to piece together concurrent events. I have, therefore, organized my observations into four distinct phases through which all *Crassostrea virginica* pass during metamorphosis. My observations of metamorphosis are consistent with those of previous researchers. Cole (1938) and Jackson (1888) noted rotation of the organs in an anterior-dorsal direction during metamorphosis of *Ostrea edulis*. Several authors (Bayne, 1971; Hickman and Gruffydd, 1971; Galtsoff, 1964; Cole, 1938) have reported the reduction of the velum and location of the velum remnants in *Mytilus edulis, C. virginica* and *O. edulis*. At no time did I observe the velum being cast off or swallowed as has been suggested by some authors (Galtsoff, 1964; Sigerfoos, 1907). However, sometimes an oyster that had the velum extended would close too quickly in response to an external stimulus. This often resulted in the oyster cutting off the velum with it's shells.

The persistence of the foot for a longer time than the velum has also been observed previously (Galtsoff, 1964;

Cole, 1938). Galtsoff (1964) stated that the eyespots broke up into irregular clumps of pigment. The dorsal movement and elongation of the eyespot into the eyestreak has not been reported previously. My observation that the labial palps are pigmented in those oysters in which the velum was pigmented, corroborates the conclusions of Galtsoff (1964), Quayle (1951), and Cole (1938) that the labial palps develop from the apical portion of the velum.

The speed at which metamorphosis occurs varies considerably between individuals. Some oysters may metamorphose to juveniles in less than 24 h while others of the same cohort may spend several days in each phase before becoming juveniles. On average, however, those oysters that complete metamorphosis successfully do so within the first 1 to 3 d post-settlement. Those oysters that fail to metamorphose may remain in the settler or prodissoconch postlarva phases for many days before dying.

I observed no abnormal development in oysters that survived exposure to either hypoxic or microxic treatments. Abnormal development has been reported in bivalve larvae grown in low oxygen. For instance, Morrison (1971) observed that eggs of *Mercenaria mercenaria* exposed to 0.34 mg O_2 l^{-1} (5% of air saturation at 25°C and 28-30 ppt) develop to the trochophore stage but do not grow shells. *Mytilus edulis* larvae, developing to the prodissoconch larval stage following 60 h exposure to 0.6 mg O_2 l^{-1} (1.38 kPa pO_2, 7% of

air saturation at 15°C and 31 ppt), do not grow shells (Wang and Widdows, 1991). Based on this observation Wang and Widdows (1991) suggested that low oxygen may interfere with the shell gland or shell secretion. If low oxygen does interfere with shell growth, and if shell growth and morphogenesis of organs are linked, this factor may have contributed to the lack of dissoconch postlarvae and juveniles in microxic treatments.

Although development was normal, I did observe arrested development after even short-term exposures to low oxygen. Oysters in normoxic treatments passed quickly through the first three phases of metamorphosis and juveniles appeared as early as 24 h post-settlement. Following 2 and 3 d exposures to microxia and 3 d exposures to hypoxia development to the juvenile phase was generally delayed until several days into the recovery period. Final proportions of juveniles, though, were the same for normoxic and hypoxic treatments, in both the continuous and recovery experiments.

Short term low oxygen exposures also delay development in other species of bivalves. Wang and Widdows (1991) report delayed development of *M. edulis* embryos to prodissoconch larvae during and following 60 h exposures to 0.6 and 1.3 mg O_2 l^{-1} (1.38 kPa pO_2 or 7% of air saturation, and 3.16 kPa pO_2 or 15% of air saturation, respectively). Veliconch larvae of *M. edulis* had eyespot development

delayed by 1, 2, and more than 6 d when exposed continuously to oxygen treatments of 2.4 mg O_2 l^{-1} (5.91 kPa pO_2, 29% of air saturation), 1.3 mg O_2 l^{-1}, and 0.6 mg O_2 l^{-1}, respectively.

The 14 d period post-settlement appears to be one of the most critical in the life cycle of the oyster. I found that approximately 50% of the control oysters died by 14 d post-settlement with the majority of the mortality occurring during the second week. Treatments in which oysters were exposed to short-term hypoxia showed similar mortality trends. Initial mortality of *C. virginica* in the field is also high. Roegner (1991) reported 73% mortality within a week of settlement. Mortality rates of eastern oysters that survive the first 7 to 14 d post-settlement, however, are less than 4% per week (Roegner, 1991).

All exposures to hypoxia, both short term and continuous, and 1 d exposure to microxia had little effect on the median mortality time or final total mortality, compared to controls. Microxic treatments of longer than 1 d duration, however, did increase mortality. During continuous exposure to microxia, oysters had a median mortality time of 87 h. This time is in good agreement with the median mortality time of 84 h already reported (Chapter 2). Widdows *et al.* (1989) report microxic median mortality times of 11, 18, and 51 h for oyster prodissoconch, veliconch, and pediveliger larvae, and 150 h for juveniles

16 mm in shell height. The median mortality time of post-settlement oysters is consistent with the trend of increasing tolerance of low oxygen conditions with developmental stage and body size. The median mortality times and relative insensitivity of post-settlement oysters to short term exposures suggest that they, like larvae and adults, are capable of anaerobic metabolism, but only for a limited time.

There was high variation in speed of development and proportion of mortality among replicates both in the continuous and recovery experiments, especially in hypoxic and microxic treatments. Gallager *et al.* (1986) observed a positive correlation between egg quality, measured as lipid content, and the proportion of *C. virginica* and *M. mercenaria* larvae completing metamorphosis. Borsa et al. (1992) report a positive relationship between heterozygosity and survival of microxic stress in the bivalve *Ruditapes decussatus*. If egg quality and/or heterozygosity of the several cohorts of larvae that I used differed, these factors may, at least partially, explain the variations observed between replicates.

Growth rates of dissoconch postlarvae and juveniles, during recovery from short term exposures to low oxygen, were not different from the growth rates of control oysters, as shown by the lack of statistical difference between slopes of the regressions. Growth rates were between 11 and

20 μm d^{-1}. Elevations of some of the regressions were significantly different from each other, indicating that growth rates prior to the recovery period varied. For instance, oysters that experienced 2 and 3 d exposures to microxia had lower growth regression elevations during recovery than did the controls. The oysters in microxic treatments, therefore, had slower growth rates during the exposure period.

Short term exposures to low oxygen appear to have no permanent effect on growth rates of larvae of other bivalve species. Morrison (1971) observed that the growth rates of *M. mercenaria* larvae varied directly with dissolved oxygen level but that growth rates became normal when transferred to normoxia following exposures to 1.0 mg O_2 l^{-1} (14% of air saturation) for up to 6 d. Growth rates of *M. edulis* veliconch larvae equaled control growth rates after 2 d of recovery from 6 d exposures to 0.6 mg O_2 l^{-1} (Wang and Widdows, 1991).

Metamorphosis is a stage in the life cycle of bivalves that is rarely studied. It is demonstrated here, however, that the 14 d period following settlement is especially critical to recruitment. Low oxygen conditions, in particular those that are microxic and last longer than 24 h, exacerbate mortality and have detrimental effects on the development and growth of post-settlement oysters. Intrusions of microxic water onto oyster beds, therefore,

may limit recruitment into the adult population by slowing development and growth, and by increasing mortality.

Figure 5. *Crassostrea virginica*. The four phases of metamorphosis in the oyster. (A) Settler. (B) Prodissoconch postlarva. (C) Dissoconch postlarva. (D) Juvenile. *a*, anus; *d*, dissoconch edge; *e*, eyespot; *es*, eyestreak; *f*, foot; *g*, gills; *gr*, gill rudiments; *ilp*, inner labial palps; *m*, mantle edge; *olp*, outer labial palps; *p*, prodissoconch edge; *pam*, posterior adductor muscle; *v*, velum; *vr*, velar remnant.

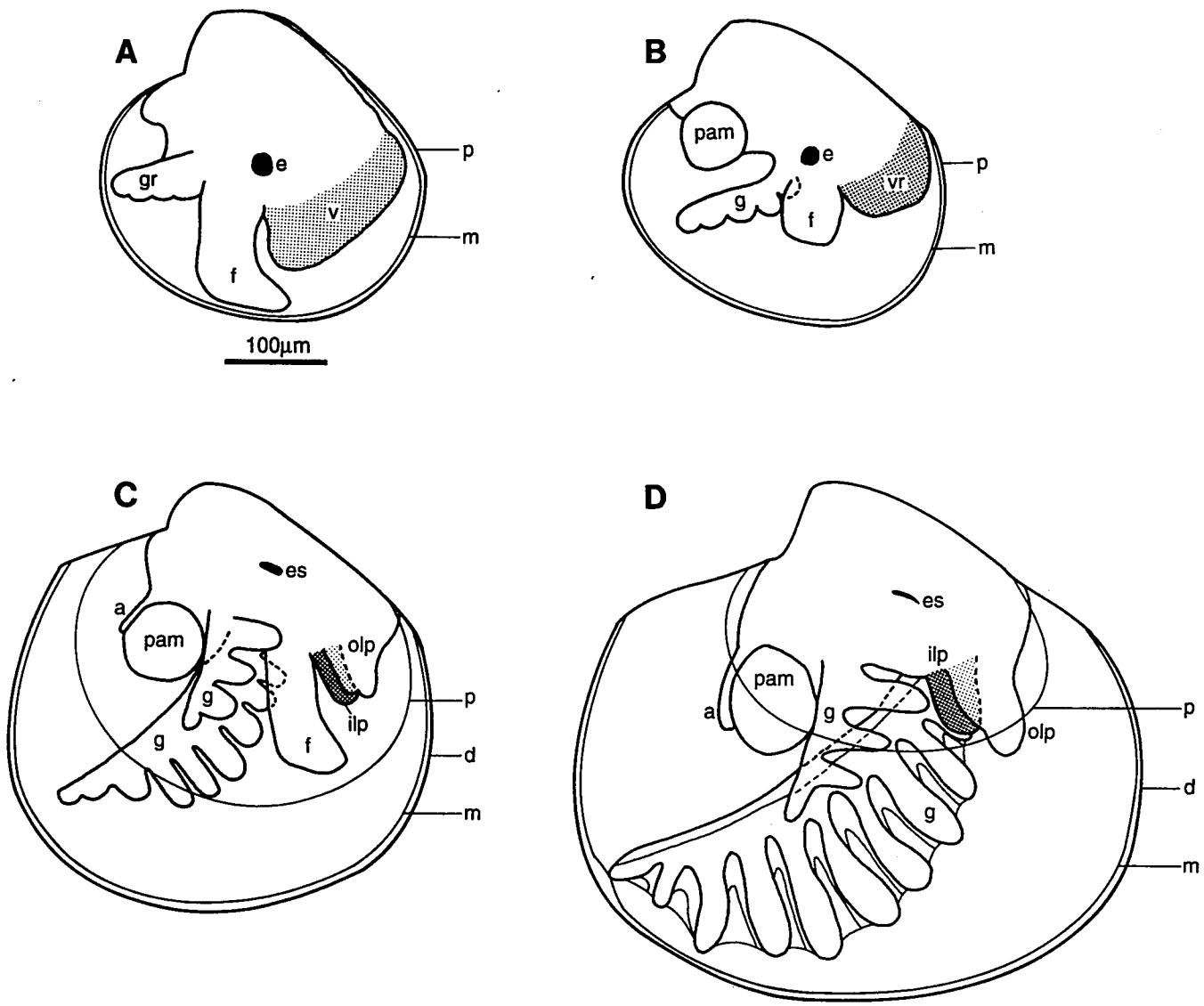

A
e
gr
v
f
p
m
100µm
B
pam
e
g
f
vr
p
m
C
es
a
pam
olp
g
g
f
ilp
p
d
m
D
es
ilp
pam
a
g
olp
p
d
g
m

Figure 6. *Crassostrea virginica*. Percentage of four metamorphic phases after different periods of continuous exposure to three oxygen concentrations: normoxia (7.3 mg O_2 l^{-1}), hypoxia (1.5 mg O_2 l^{-1}), and microxia (< 0.07 mg O_2 l^{-1}). (Means + SD.) MMT = median mortality time.

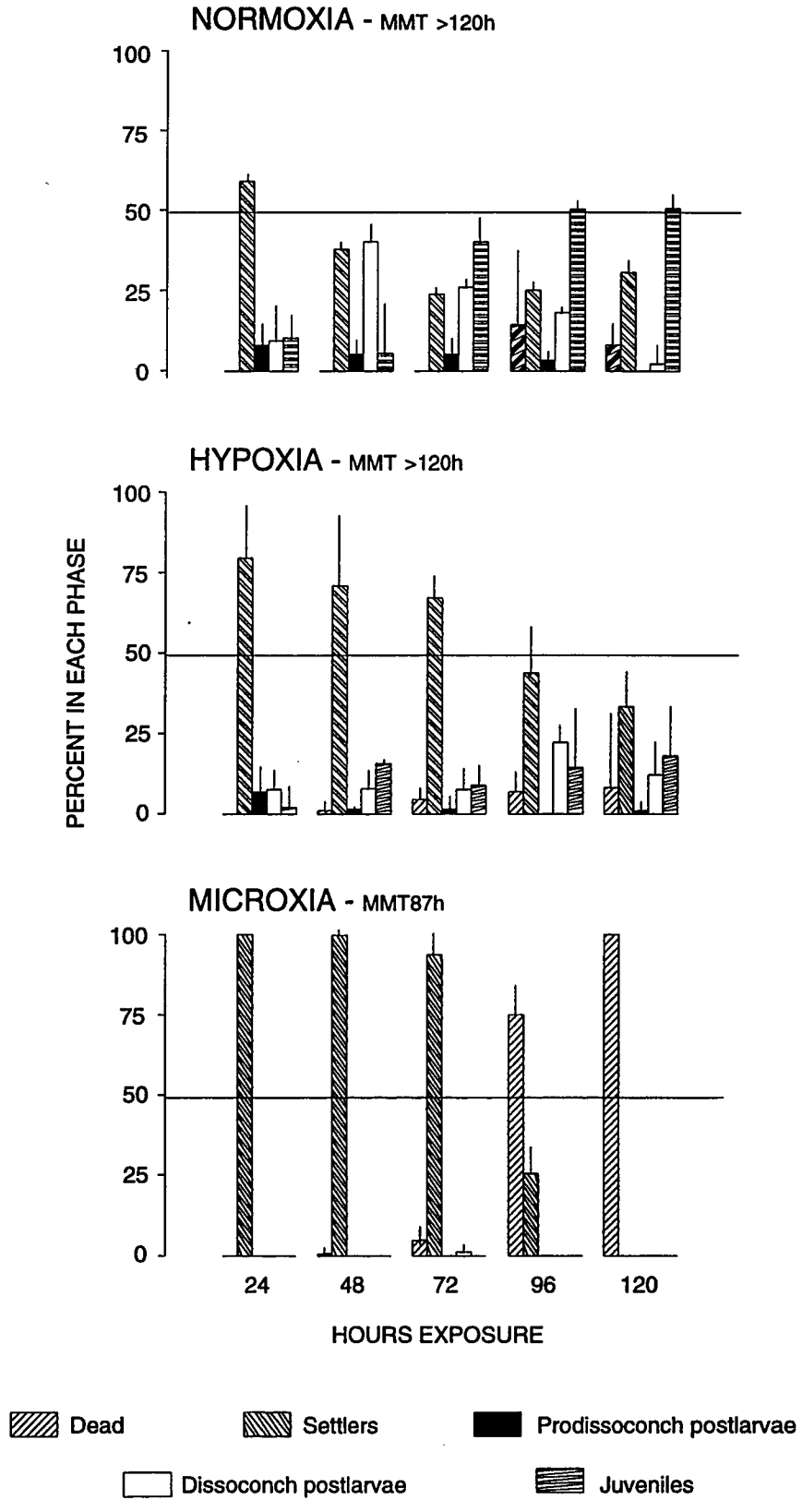

NORMOXIA - MMT >120h
HYPOXIA - MMT >120h
MICROXIA - MMT87h
PERCENT IN EACH PHASE
100
75
50
25
0
24
48
72
96
120
HOURS EXPOSURE
Dead
Settlers
Prodissoconch postlarvae
Dissoconch postlarvae
Juveniles

Figure 7. *Crassostrea virginica*. Percentage of four metamorphic phases during recovery from 1, 2, or 3 d exposures to normoxia (7.3 mg O_2 l^{-1}), hypoxia (1.5 mg O_2 l^{-1}), and microxia (< 0.07 mg O_2 l^{-1}). (Means + SD.) MMT = median mortality time.

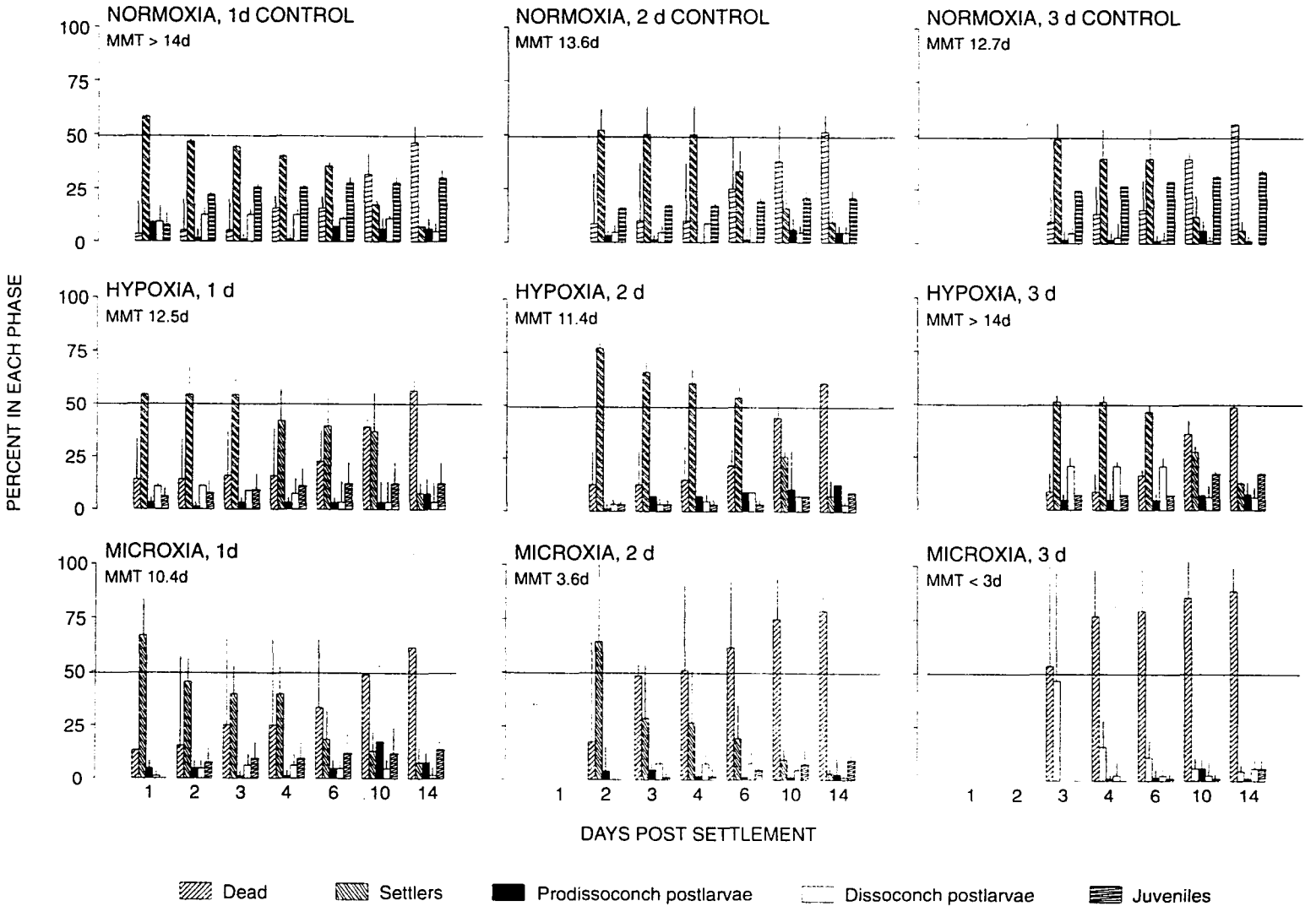
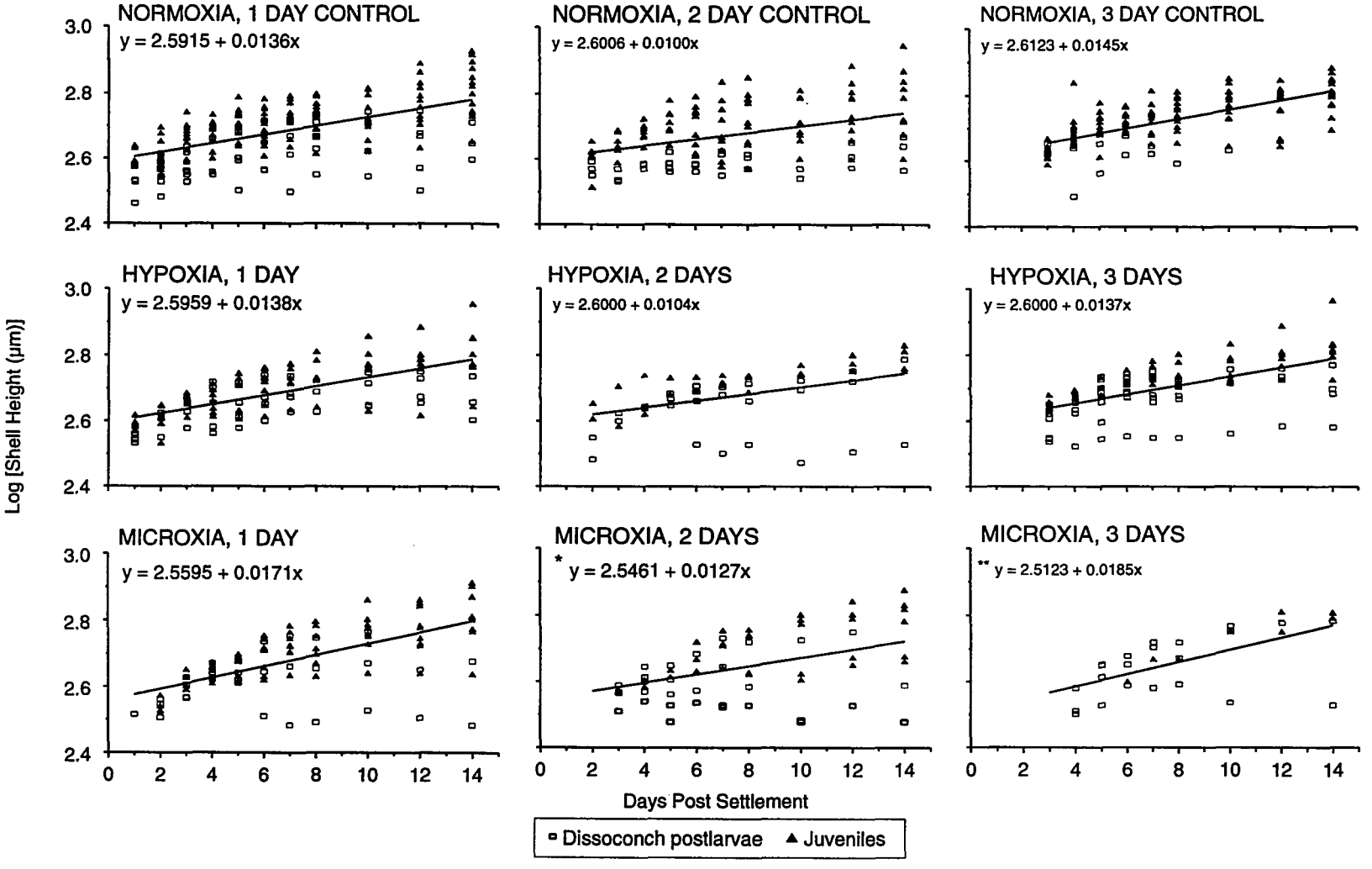

NORMOXIA, 1d CONTROL
MMT > 14d
NORMOXIA, 2 d CONTROL
MMT 13.6d
NORMOXIA, 3 d CONTROL
MMT 12.7d
HYPOXIA, 1 d
MMT 12.5d
HYPOXIA, 2 d
MMT 11.4d
HYPOXIA, 3 d
MMT > 14d
MICROXIA, 1d
MMT 10.4d
MICROXIA, 2 d
MMT 3.6d
MICROXIA, 3 d
MMT < 3d
PERCENT IN EACH PHASE
100
75
50
25
0
1 2 3 4 6 10 14
DAYS POST SETTLEMENT
Dead
Settlers
Prodissoconch postlarvae
Dissoconch postlarvae
Juveniles

Figure 8. *Crassostrea virginica*. Log_{10} of shell height of dissoconch postlarvae and juveniles following 1, 2, and 3 d exposures to normoxic (7.3 mg O_2 l^{-1}), hypoxic (1.5 mg O_2 l^{-1}), and microxic (< 0.07 mg O_2 l^{-1}) treatments. *, elevation of regression significantly lower ($P \leq 0.05$) than control; **, elevation significantly lower ($P \leq 0.05$) than both hypoxic treatment and control.


NORMOXIA, 1 DAY CONTROL
y = 2.5915 + 0.0136x
NORMOXIA, 2 DAY CONTROL
y = 2.6006 + 0.0100x
NORMOXIA, 3 DAY CONTROL
y = 2.6123 + 0.0145x
HYPOXIA, 1 DAY
y = 2.5959 + 0.0138x
HYPOXIA, 2 DAYS
y = 2.6000 + 0.0104x
HYPOXIA, 3 DAYS
y = 2.6000 + 0.0137x
MICROXIA, 1 DAY
y = 2.5595 + 0.0171x
MICROXIA, 2 DAYS
* y = 2.5461 + 0.0127x
MICROXIA, 3 DAYS
** y = 2.5123 + 0.0185x
Log [Shell Height (µm)]
3.0
2.8
2.6
2.4
0
2
4
6
8
10
12
14
Days Post Settlement
Dissoconch postlarvae
Juveniles

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Chapter 4

FEEDING ABILITY DURING SETTLEMENT AND METAMORPHOSIS IN THE OYSTER *CRASSOSTREA VIRGINICA* (GMELIN, 1791) AND THE EFFECTS OF HYPOXIA ON POST-SETTLEMENT INGESTION RATES

Abstract

I observed the feeding ability of oyster (*Crassostrea virginica*) larvae during settlement behavior and metamorphosis. I also examined the effects of hypoxia (1.9 mg O_2 l^{-1}, 25% of air saturation) and microxia (< 0.4 mg O_2 l^{-1}, < 5% of air saturation) on ingestion rates of post-settlement oysters. I tested the null hypothesis that there were no effects of oxygen treatment, length of exposure, or interaction of treatment and exposure time on ingestion rates. Velar feeding occurred during the searching and crawling stages of settlement behavior, but not during cementation. In newly settled larvae, 16% engaged in velar feeding while 84% did not feed. All later metamorphic phases fed, although the mode of particle capture was unclear. Under normoxic conditions mean rates of cell ingestion by post-settlement oysters (268 to 651 μm in shell height) ranged from 600 to 3,000 cells h^{-1} $individual^{-1}$. In response to hypoxic treatments, post-settlement oysters with shells heights of 469 ± 103 μm and 651 ± 131 μm maintained their normoxic rates of ingestion but oysters with shell heights of 436 ± 60 μm reduced their ingestion rates to 54%-61% of normoxic rates. These oyster sizes differed in the degree of gill proliferation. In response to microxic treatments, ingestion rates were 1% - 14% of normoxic rates

and decreased with body size. I conclude that oysters have the ability to feed at nearly all stages of settlement and metamorphosis and that hypoxic conditions will affect the feeding activities of only the youngest post-settlement oysters while microxic conditions will affect all post-settlement oysters.

Introduction

Larvae of the eastern oyster, *Crassostrea virginica* (Gmelin, 1791), develop and grow in the plankton until the final larval stage, the pediveliger, permanently attaches to a substrate (Galtsoff, 1964). Following settlement, the pediveliger begins to metamorphose into the juvenile which involves major changes in both form and function (Fioroni, 1982). Of particular note is the transition from velum to gill as the feeding organ (Yonge, 1926). Little is known about feeding during this transition. Thus, the first objective of my study was to describe the feeding ability of oysters throughout the settlement and metamorphic process.

Chesapeake Bay, as well as some other coastal embayments and estuaries, exhibits annual summer oxygen depletion as a result of stratification, and other factors (Kuo and Neilson, 1987). Oxygen depletion is usually restricted to the deep waters below the pycnocline. Water set-up by wind stress, however, may tilt the pycnocline and irrigate the shallow areas, where oyster reefs occur, with deep hypoxic and microxic water. The pycnocline may remain tilted for several hours to 2 or 3 d (Breitburg, 1990; Sanford *et al.*, 1990). Oxygen depletion and the intrusion of hypoxic water onto the shallow banks of the Chesapeake Bay often coincides with the period of spawning, larval

settlement and metamorphosis of the oyster. Widdows *et al.* (1989) studied the effects of hypoxia and microxia on rates of ingestion by oyster larvae. Under hypoxic conditions there is a marked decline in the proportion of larvae feeding and in rates of ingestion (Widdows *et al.*, 1989). In Chapter 2 I reported a reduction in and complete lack of growth of metamorphosing oysters when exposed to hypoxia and microxia, respectively. I hypothesized that the failure to grow was, at least partially, the result of decreased feeding rates. The second objective of my study, therefore, was to examine the effects of low oxygen on the ingestion rates of post-settlement oysters. I tested the null hypothesis that there were no effects of oxygen treatment, length of exposure, or interaction of treatment and exposure time on ingestion rates.

Materials and Methods

Feeding observations

Pediveliger larvae of the eastern oyster, *Crassostrea virginica*, were obtained from the Virginia Institute of Marine Science hatchery at Gloucester Point, VA. Feeding ability was observed during settlement, which lasts from minutes to hours and consists of a searching stage, a crawling stage, and cementation (for a review, see Prytherch, 1934; Galtsoff, 1964; Cranfield, 1973). Groups of oyster larvae were placed in a rafter cell (1 ml) with seawater. Settlement behavior was induced with 2.5×10^{-5} M L-3,4 dihydroxyphenylalanine (L-Dopa) (Coon and Bonar, 1985; Salas *et al.*, 1989).

To study feeding ability during metamorphosis, oyster larvae were allowed to attach to substrates to initiate metamorphosis. Frosted Mylar acetate settlement substrates were held in seawater for at least 2 d prior to each experiment to develop a settlement-inducing bacterial coating (Fitt *et al.*, 1990). Oyster larvae were exposed to a conditioned substrate unit for 1 h. Larvae that had not settled and attached within that time were rinsed from the substrate unit. Attached oysters were removed from the substrate unit with a razor blade so that they could be manipulated on an individual basis. I compared the growth

and morphology of oysters which had been removed from the substrate and those that had not. I found that there was no difference in growth patterns or morphology until the oysters reached approximately 700 μm. At this size the oyster shells began to cup because they were not growing on a flat substrate.

Feeding ability was noted in four metamorphic phases, as described in Chapter 3 (Fig. 9). Briefly, **settlers** have attached to the substrate but otherwise retain all larval characteristics; both velum and foot are intact and active (Fig. 9A). A rudiment of the inner demibranch of left gill is visible. In **prodissoconch postlarvae**, the velum has degenerated to a velar remnant and the gill has proliferated (Fig. 9B). There is no shell growth beyond the prodissoconch. **Dissoconch postlarvae** have completely resorbed the velum but still have a foot (Fig. 9C). Both the right and left gills are visible and the labial palps are active. Oysters in this phase have new shell growth at the ventral edge of the prodissoconch. In **juveniles**, metamorphosis is complete; all larval organs are lost (Fig. 9D). The descending and ascending limbs of the inner demibranch of the left gill are visible. Juveniles appeared 48 ± 29 (S.D.) h post-settlement with shell heights of 410 ± 40 μm. Settlers were observed while still attached to the substrate, and the other phases were observed after removal.

The oysters were maintained until use in glass dishes in filtered (0.45 μm) seawater of 20 ppt salinity at an ambient temperature of 24°C. Water was changed daily and the oysters were fed the flagellate *Isochrysis galbana* at a concentration of 20,000 cells ml^{-1}.

Groups of oysters at different stages of settlement and metamorphosis were placed in a rafter cell with seawater, and fluorescent carboxylate microspheres (Polysciences Fluoresbrite, 4.5 μm diameter) were added at a concentration of 50,000 microspheres ml^{-1}. To enhance ingestion, the microspheres were conditioned in *I. galbana* extract prior to use. Oysters were observed for possible feeding currents, and associated microsphere movement, using a dissecting microscope. After 30 min in the microsphere solution, the rafter cell was transferred to a Zeiss IM 35 inverted microscope equipped for epifluorescence. The presence or absence of microspheres in the guts of individuals was noted. All oysters examined were measured from the umbo to the distal shell margin (shell height) using a compound microscope fitted with an ocular micrometer.

Ingestion Rates

Treatment beakers of seawater were continuously bubbled with either air, a mixture of oxygen and nitrogen, or nitrogen. The target oxygen concentrations were 7.5 mg O_2

l^{-1} (100% of air saturation), 1.9 mg O_2 l^{-1} (25% of air saturation), and less than 0.4 mg O_2 l^{-1} (< 5% of air saturation). These treatments are referred to as normoxia, hypoxia, and microxia, respectively. Oxygen concentrations were measured with a Strathkelvin Instruments (SI) oxygen sensor (1302) coupled to a SI oxygen meter (781) and chart recorder. The oxygen sensor was calibrated with air-saturated water (100% of air saturation) and a zero oxygen solution of sodium borate and crystalline sodium sulfite. Normoxic, hypoxic, and microxic treatments were consistently maintained at 90-100%, 24-28%, and 3-5% of air saturation.

Ingestion rates of post-settlement oysters exposed to normoxia, hypoxia, and microxia, were determined as follows. Pediveliger larvae were exposed to a conditioned substrate overnight. Larvae that had not settled and attached within that time were rinsed from the substrate. The substrates, oysters attached, were maintained in glass dishes until use. When the oysters had reached the desired size, the substrate was cut into 2 x 8 cm pieces. Dead oysters and oysters of other than the desired size were removed from the substrate units. The number of oysters on a unit were estimated from 6 counts of 1 cm^2, each.

Algae, *I. galbana*, was added to seawater in covered beakers (200 ml) to a concentration of 100,000 cells ml^{-1}. This high algae concentration was chosen because decreases in concentration were more easily detected over time. The

beakers were continuously bubbled with gases, beginning 3 h prior to the experiments. Substrate units with attached oysters were placed in 9 separate beakers, 3 for each oxygen treatment (3 replicates). Conditioned substrate units without attached oysters were placed in 3 beakers to serve as controls for each of the 3 oxygen treatments. The substrate units were placed in the beakers so that they were standing up diagonally, with the attached oysters facing downward and no bubbles disturbing them.

Time 0 cell counts were taken immediately upon placing the substrate units in the beakers. Samples of 5 ml were removed from each beaker and diluted to 20 ml with Coulter Isoton II electrolyte solution in Coulter Accuvette II disposable sample vials. Cells were counted using a Coulter Counter Model TA equipped with a 100 μm aperture tube and set to draw 2 ml. Three cell counts were made for each 5 ml sample. Cell counts were corrected for dilution and background count.

Cell counts were taken at 0, 6, 18, and 24 h after initiation of the experiment. One hour prior to the 18 h sample, water and algae were added to bring the water volume and cell concentration back to 200 ml and 100,000 cells ml^{-1}, respectively. Following the 24 h sample, the substrate units were removed from the beakers and 15 oysters on each were measured.

The entire experiment was repeated three times using oysters with shell heights of 436 ± 60 μm (S.D., n = 135), 469 ± 103 μm (S.D., n = 135), and 651 ± 131 μm (S.D., n = 135). Although all 3 sizes were juveniles, they differed in the extent of gill development and proliferation. For convenience, they are hereafter referred to by their mean sizes. The numbers of oysters in each beaker were 878 to 1804, 257 to 696, and 320 to 488, respectively. Ingestion rates of oysters with shell heights of 268 ± 36 μm (S.D., n = 45) were determined for normoxic treatments only. These experiments used 267 to 599 oysters. Ingestion rates were calculated for the first and last 6 h periods of the experiments according to Coughlan (1969) and Sprung (1984):

$$F = \frac{v}{n}\left(\frac{\ln C_0 - \ln C_t}{t} - A\right)$$

$$A = \frac{\ln C_{0*} - \ln C_{t*}}{t}$$

$$I = FC$$

where F = filtration rate (ml h^{-1} $individual^{-1}$), n = number of oysters in beaker, v = average volume of beaker during clearance rate measurement (190 ml), C_0 = cell concentration at Time 0 or Time 18 (cells ml^{-1}), C_t = cell concentration at Time 6 or Time 24 (cells ml^{-1}), C_{0*} and C_{t*} = cell concentrations in control beakers (cells ml^{-1}), t = time over

which clearance rate was measured (6 h), A = changes in the control beakers, I = ingestion rate (cells h^{-1} $individual^{-1}$), and C = the mean cell concentration during ingestion rate measurement (cells ml^{-1}).

A two-way analysis of variance was performed for each oyster size group (except 268 μm) to test the null hypotheses that there were no effects of oxygen treatment, length of exposure (0-6 h or 18-24 h), or interaction on ingestion rate. If a null hypothesis was rejected, Tukey's multiple comparison test was used to identify differences between the specific oxygen treatments and exposure times. Statistical analyses were conducted using Minitab software.

In addition to the ingestion rate experiments, the proportion of oysters feeding during exposure to the three oxygen treatments was assessed. Oxygen treatments were produced as described. Post-settlement oysters were obtained as described and the substrate was cut into 1 x 8 cm pieces. Algae was added to seawater in covered beakers to concentrations of 80,000 cells ml^{-1}. Substrate units with attached oysters were placed in 12 separate beakers, 4 for each oxygen treatment.

At Time 0, microspheres were added to 3 beakers, 1 from each oxygen treatment, to a concentration of 20,000 microspheres ml^{-1} for a final concentration of 100,000 particles ml^{-1}. After 1 h the substrate units were removed from those 3 beakers and fixed in ethanol. Beads were added

and oysters removed from 1 beaker per oxygen treatment at 5 and 6 h, 18 and 19 h, and 23 and 24 h after initiation of the experiment. At 17 h algae was added to the 6 beakers still in use to bring the cell concentration back to 80,000 cells ml^{-1}. Oysters removed from the beakers were immediately fixed in ethanol. The presence or absence of microspheres in the guts of 150 fixed oysters was noted for each of the 12 oxygen treatment-exposure time combinations. Oysters had to have an indistinguishable number of microspheres in the guts to be counted as having ingested microspheres. Therefore, oysters with, for instance, 1 to 5 microspheres in the gut were not counted as having ingested microspheres. The shell heights of twenty oysters on each substrate unit were measured. The entire experiment was repeated two times using oysters with shell heights of 277 ± 31 μm (S.D., n = 240) and 419 ± 72 μm (S.D., n = 240). These oyster sizes differed in metamorphic phase.

Results

Feeding observations

Feeding ability was observed during settlement behavior. Pediveliger oyster larvae (282 ± 10 μm shell height, S.D., n = 10) in the searching stage of settlement swam near the substrate with both the velum and foot extended. All larvae observed in this stage of settlement behavior ingested microspheres and retained them for several hours, as indicated by fluorescence in their guts. When larvae began the crawling stage of settlement, the velum was generally withdrawn. During the early stages of crawling, however, some larvae extended the velum for periods of a few seconds and these animals ingested microspheres. When microspheres were not provided until the later stages of crawling behavior, and the larvae no longer extended the velum, microspheres were not ingested. During the crawling stage, some larvae left the substrate and resumed swimming and searching behavior. Larvae that did so also resumed feeding. In both the searching and crawling stages of settlement behavior, microspheres were seen revolving in the area of the presumptive crystalline style sac. Fecal masses containing microspheres were periodically ejected from the anus. No microspheres were ingested or ejected during the process of cementation to the substrate.

Feeding ability was also examined during metamorphosis. After attachment to the substrate, some settlers (285 ± 20 μm, S.D, n = 10) continued to extend the velum and the velar cilia continued to beat for approximately 5 h post-settlement (Fig. 9A). These individuals ingested microspheres and retained them for several hours, as indicated by the fluorescence of their guts. Of the 200 oysters examined at the settler phase of metamorphosis, 16% exhibited extension of the velum and ingestion of microspheres. In settlers that did not extend the velum, microspheres were observed entering the visceral cavity ventrally, and exiting posteriorly but microspheres did not appear to come into contact with the rudimentary gill.

All oysters observed at the prodissoconch postlarva phase (approximately 18 - 29 h post-settlement) of metamorphosis ingested microspheres (n = 10, 294 ± 6 μm, S.D.) (Fig. 9B). The degenerated velar remnant was never extended and there was only erratic beating of the remaining cilia. Again, some microspheres entered the visceral cavity ventrally, and exited posteriorly. The exact path of microspheres that were ingested could not be discerned but it appeared that microspheres, whether accepted or rejected, did not come into contact with the gill.

Microspheres were also ingested by all dissoconch postlarvae (n = 10, 333 ± 24 μm, S.D.)(approximately 29 - 48 h post-settlement) and juveniles (n = 15, 364 ± 19 μm,

S.D.)(over 48 h post-settlement) examined (Fig. 9C and 9D). In both of these phases, some microspheres entered and exited the visceral cavity, as described before. In these phases, however, accepted microspheres appeared to come into contact with the 3 most anterior filaments of the right and left gills. Pseudofeces were noted outside the shell margin, at the distal end of the left gill. These pseudofeces are similar to those of adults in that they are particles which have been rejected and not ingested but, they do not appear to be formed by the labial palps. Fecal masses containing microspheres were ejected periodically from the anus. In all phases of metamorphosis, microspheres were seen revolving in the crystalline style sac.

Ingestion rates

Mean normoxic rates of cell ingestion by post-settlement oysters, 268 to 651 μm in shell length, ranged from 600 to 3,000 cells h^{-1} $individual^{-1}$, increasing with body size (Fig. 10, A-D). Oxygen treatment had a significant (two-way ANOVA; 436 μm, $P = 0.001$; 469 μm, $P = 0.017$; 651 μm, $P = 0.000$) effect on the ingestion rates of the three sizes of oysters in which this parameter was examined. For oysters of 436 μm shell height (juveniles), ingestion rates were significantly (Tukey's test, $P = 0.001$) different in all three oxygen treatments (Fig. 10B). Mean hypoxic ingestion rates were 61% and 54% of normoxic rates between

Time 0 and 6 and between Time 18 and 24, respectively. Microxic rates were 7% (Time 0 to 6) and 14% (Time 18 to 24) of those in normoxia. Exposure time and the interaction of oxygen treatment and time were not significant (two-way ANOVA, $P = 0.944$) for this size class.

The ingestion rates of oysters with mean shell heights of 469 μm (juveniles) were not significantly different in normoxic and hypoxic treatments (Tukey's test, $P > 0.50$) (Fig. 10C). Microxic ingestion rates, however, were significantly (Tukey's test, $P \leq 0.046$) lower than both normoxic and hypoxic rates. Mean microxic ingestion rates were 18% (Time 0 to 6) and 19% (Time 18 to 24) of normoxic rates. Again, exposure time and the interaction of oxygen treatment and exposure time were not significant (two-way ANOVA, $P = 0.717$).

In the largest size post-settlement oysters examined (651 μm, juveniles), normoxic and hypoxic ingestion rates were not significantly different (Tukey's test, $P > 0.50$) from each other and both were significantly (Tukey's test, $P > 0.001$) greater than microxic ingestion rates (Fig. 10D). Mean ingestion rates in microxic treatments were 1% (Time 0 to 6) and 3% (Time 18 to 24) of rates in normoxia. Unlike the previous size classes, the effect of exposure time and the interaction of exposure time and oxygen treatment were significant (two-way ANOVA, $P = 0.000$). Both normoxic and hypoxic ingestion rates decreased significantly (Tukey's

test, $P < 0.001$) over the 24 h exposure time. Ingestion rates of oysters in normoxic and hypoxic treatments decreased 32% and 39% respectively. Microxic ingestion rates did not change significantly (Tukey test, $P > 0.50$) over time.

The proportions of oysters feeding during 24 h exposures to normoxic, hypoxic, and microxic treatments, are shown in Fig. 11. In the group with a mean shell height of 277 μm (settlers and prodissoconch postlarvae), the proportions of oysters feeding during the 1 h intervals were approximately 64% and were similar in normoxia and hypoxia (Fig. 11A). The proportions feeding in these treatments remained fairly constant over the 24 h exposure period. In microxic treatments, 11% of the oysters fed during the first h of exposure. The proportion of feeding oysters declined to 1% by the 5-6 h sample and remained between 1% and 2% for the remainder of the experiment.

Proportional feeding of oysters with shell heights of 419 μm (dissoconch postlarvae and juveniles) was approximately 81% in both normoxia and hypoxia over the 24 h exposure, except at the 18-19 h sample when the proportion of oysters feeding in hypoxia dropped to 51% (Fig. 11B). In microxia, 9% of the oysters fed during the first hour of exposure, with the proportion dropping to 6% and 5% over the remainder of the experiment.

Discussion

The feeding ability of bivalves during settlement behavior has not been previously reported. Velar feeding occurred during the searching and crawling stages of settlement behavior in the oyster *Crassostrea virginica*. Velum-generated flow fields around searching larvae appeared to be similar to those described by Gallager (1988) for larvae of the clam *Mercenaria mercenaria* (L., 1758). Flow fields around crawling stages, and settlers engaged in velar feeding, were distorted by the proximity of the substrate.

Except for only a few hours during the settler phase, feeding is possible throughout oyster metamorphosis. Several authors have studied the morphology of bivalve larvae or metamorphosing bivalves and have concluded, based on the structure of the developing gills, that metamorphosing bivalves are not able feed (Bayne, 1965; Waller, 1981). Based on their conclusions, as well as on the descriptive works of Cole (1938), Sastry (1965), Hickman and Gruffydd (1971), and Elston (1980), it has generally been assumed that feeding does not occur for a period of several days while the velum is resorbed and the rudimentary gills proliferate and develop food grooves. Food grooves do not develop, however, until some time after metamorphosis is complete. In *C. virginica* and the clam *Venus striatula* (Da

Costa), for instance, food grooves are not observed until the bivalves are approximately 2 to 3 mm in size (Stafford, 1913; Ansell, 1962).

Some siphonate bivalves, however, are able to capture food particles before the gill is fully developed. In *Macoma balthica* (Caddy, 1969) and *Mytilus edulis* (Bayne, 1971a) cilia on the foot bring food particles into the mantle cavity where they are transported to the mouth by the developing gill and labial palps.

Despite these observations, the idea that metamorphosing bivalves do not have the ability to feed has been perpetuated in the literature. For instance, Holland and Spencer (1973), Gallager *et al.* (1986), and Rodriguez *et al.* (1990) base their rationale for studies on biochemical composition of larval and metamorphosing bivalves on the assumption that metamorphosing bivalves are unable to feed and must rely solely on stored energy reserves.

While the rationale behind the biochemical studies is questioned, the fact remains that endogenous reserves are used during bivalve metamorphosis (Holland and Spencer, 1973; Rodriguez, 1990). Whyte (1992) reports that although the fatty acid profiles of early post-metamorphic scallops *Crassadoma gigantea* (Gray) indicate food assimilation, they continue to rely on endogenous reserves as well. Thus, it appears that feeding alone may be insufficient to meet the high energy demands of metamorphosis and early growth.

Inhalant and exhalent currents, which were noted in all phases of metamorphosis, are observed during the development of other species of bivalves as well. Waller (1981) suggests that inhalant and exhalent currents are present in the pediveliger larvae of the oyster *Ostrea edulis* (Linné). Ansell (1962) states that the developing gill filaments of *V. striatula* create water currents that impinge on the labial palps but the filaments do not collect and deliver food materials to the palps as gill filaments do in the adult.

The mode of particle capture, transport, and selection was unclear in metamorphosing oysters, especially in the prodissoconch postlarva phase. Microsphere contact with the gills and formation of pseudofeces was not observed until the dissoconch postlarva phase of metamorphosis. It is not surprising, however, that the feeding mechanism of these bivalves differs from that of adults. Declerck (1991) suggests that filter feeding mechanisms in small gastropods are different than those of adults because the Reynolds number of the flow around the gills is much lower. At low Reynolds numbers viscous forces are much more important than inertial forces and so hydraulic resistance at the entrance to the mantle cavity and between the gill filaments is very large. The high metabolic cost of generating feeding currents at low Reynolds numbers may contribute to the reliance on endogenous reserves in post-settlement bivalves.

Mircospheres were retained for several hours in those oysters that ingested them. This is in contrast to what is described by Gallager (1988) and Robinson (1981). They report that *Mercenaria mercenaria* pediveliger larvae pass microspheres through the gut as fast as the particles are ingested. I observed no post-ingestion selection of particles.

The normoxic rates of ingestion, 600 - 3,000 cells h^{-1} $individual^{-1}$, by post-settlement oysters compare favorably with previously reported rates. Ingestion rates of 440 and 150 cells h^{-1} $individual^{-1}$ are reported for mussel *Mytilus edulis* (Linné, 1758) pediveliger larvae (245 and 278 μm) (Sprung, 1984; Wang and Widdows, 1991). Widdows *et al.* (1989) report an ingestion rate for *C. virginica* pediveligers (300 to 376 μm) of 240 microspheres h^{-1} $individual^{-1}$. Post-settlement *O. edulis* with shell heights of 500 μm have ingestion rates of about 5,000 cells h^{-1} $individual^{-1}$ (Walne, 1972) and the oyster *Crassostrea gigas* (Thunberg, 1793), at 1 to 3 d post-settlement, has ingestion rates from 2,000 to 5,700 cells h^{-1} $individual^{-1}$ (Gerdes, 1983).

In response to hypoxia, the larger post-settlement oysters examined (469 and 651 μm) maintained their normoxic rates of ingestion. Adult bivalves also maintain or even increase their rates of ingestion in response to hypoxia. For instance, *M. edulis* (Bayne, 1971b) and the clam *Arctica*

islandica (Taylor and Brand, 1975) have filtration rates that are maintained and increased, respectively, down to about 2.1 mg O_2 l^{-1} (40 mm Hg and 5 kPa pO_2, 25% of air saturation at 15°C) . An increase in ventilation rate, as indicated by an increase in ingestion and filtration rates, results in a greater volume of water passing over the gills to compensate for the low oxygen concentration.

Sensitivity to hypoxia appears to decrease with increasing body size in post-settlement oysters. While the larger post-settlement oysters maintained their ingestion rates in response to hypoxia, the smaller oysters (436 μm) did not. Larval bivalves exhibit the opposite pattern; sensitivity to hypoxia increases with increasing body size. Prodissoconch larvae (114 and 150 μm) and veliconch larvae (180 μm) of *M. edulis* maintain or even increase ingestion rates, compared to normoxic controls, during the first 24 h of exposure to oxygen levels as low as 1.3 mg O_2 l^{-1} (3.16 kPa pO_2, 15% of air saturation at 15°C and 31 ppt) but pediveliger larvae (278 μm) do not (Wang and Widdows, 1991). Prodissoconch (73 - 140 μm) and pediveliger larvae (300 - 376 μm) of *C. virginica* decrease ingestion rates to approximately 50% and 4% of control rates, respectively, in response to 24 h exposures to 0.8 to 2.4 mg O_2 l^{-1} (2.2 to 6.0 kPa pO_2, 10 to 29% of air saturation at 22°C and 12ppt) (Widdows *et al.*, 1989). The relationship between hypoxic feeding rates and body size in bivalve larvae is attributed

to a reduction in metabolic rate, the result of oxygen diffusion problems associated with a decreasing surface area to volume ratio (Widdows *et al.*, 1989; Wang and Widdows, 1991). In post-settlement oysters, the trend of decreasing sensitivity with increasing size suggests that, as the gills proliferate, the surface area to volume ratio increases and oxygen diffusion becomes less of a problem.

Only in the largest post-settlement oysters examined (651 μm) did exposure time have a significant effect on ingestion rate. Both normoxic and hypoxic ingestion rates decreased approximately 35% over the 24 h period. This observation suggests that, as the gills grow, they become more efficient at particle capture and the larger post-settlement oysters are able to satiate their guts. Once the gut is full, ingestion rate is limited by the gut residence time (Sprung, 1984). This observation also suggests that the larger post-settlement oysters were slightly starved prior to the experiments.

In their response to microxia, post-settlement oysters, especially the largest examined (651 μm), resemble pediveliger oyster larvae. Oyster pediveligers slightly increase their rates of ingestion from 2 to 4% of their normoxic rates over a 24 h exposure to 0.3 - 0.7 mg O_2 l^{-1} (0.8 - 1.7 kPa pO_2, 4 to 7% of air saturation). Prodissoconch larvae, however, have microxic rates as high as 50% of normoxic ingestion rates (Widdows *et al.*, 1989).

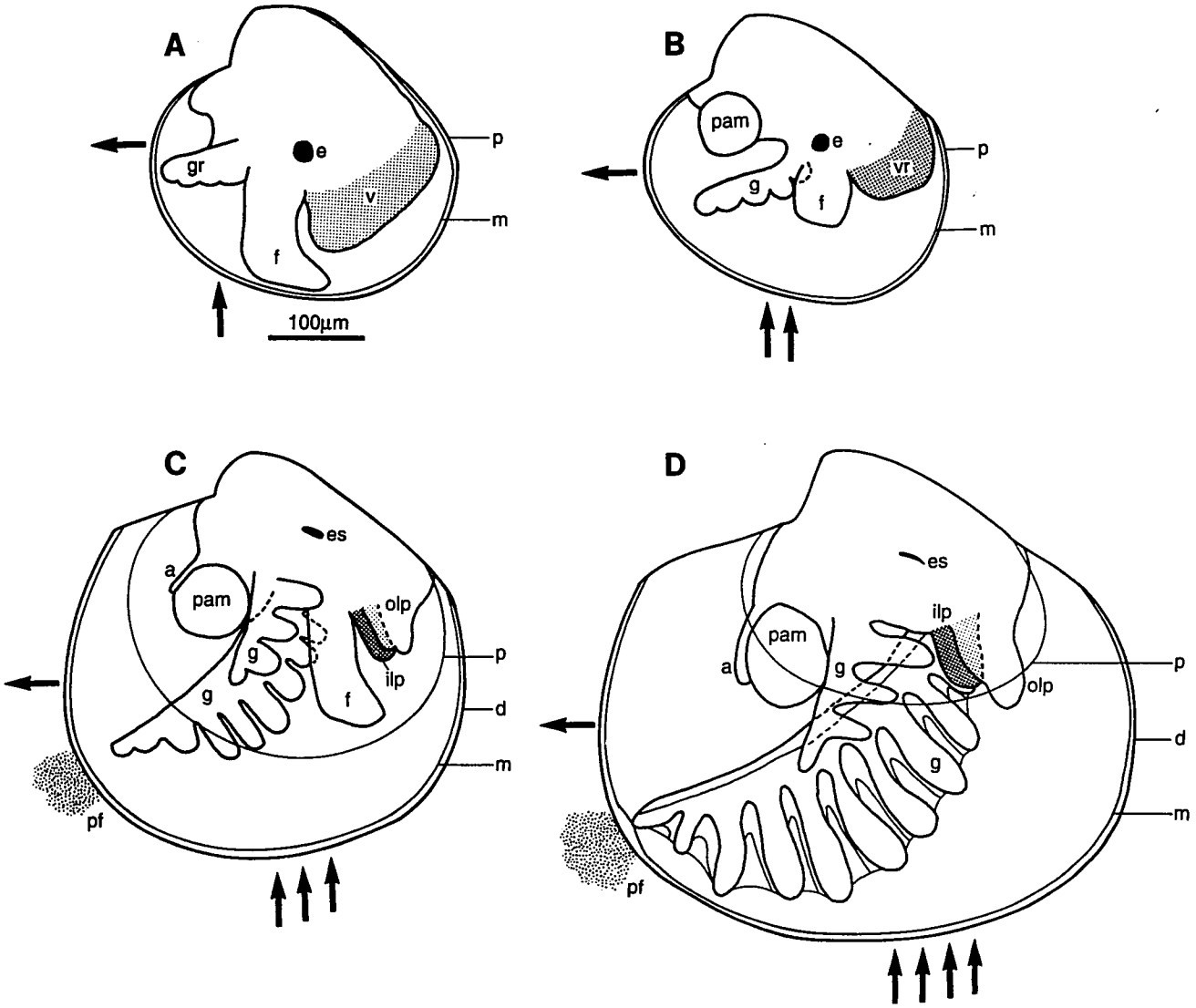
The proportions of post-settlement oysters which fed under normal and low oxygen conditions were similar to those reported for adult bivalves but markedly different from those reported for larvae. The proportions of adults of the clam *Theora fragilis* feeding after 24 h in conditions of 6.7, 2.2, and 1.3 mg O_2 l^{-1} (100%, 33%, and 19% of air saturation at 25°C and 30 ppt), are 100%, 80%, and 20%, respectively (Tamai, 1993). Fifty to 80% of pediveliger oyster larvae feed under normoxic conditions during a 10 min period but this proportion decreases to about 20% and 0% in response to 24 h exposures to hypoxia of 1.7-2.4 mg O_2 l^{-1} and 0.8-1.1 mg O_2 l^{-1}. The proportion decreases to 5% in response to microxia (0.3 - 0.7 mg O_2 l^{-1}). Only 25 to 55% of oyster prodissoconch larvae feed during a 10 min period in normoxia. At the end of 24 h exposures to hypoxic conditions, the proportions of prodissoconch larvae feeding is about 5%, and at the end of exposure to microxia the proportion is about 10% (Widdows *et al.*, 1989).

Both hypoxia and microxia have significantly negative affects on growth (Chapter 2). Post-settlement oysters in hypoxic conditions (1.5 mg O_2 l^{-1}, 20% of air saturation at 25°C and 21 ppt) grow one third as much as those in normoxia, over a period of 144 h. Post-settlement oysters in microxia (< 0.07 mg O_2 l^{-1}, < 1% of air saturation) do not grow at all. They attribute these observations to a decrease in, or cessation of, feeding. Results of the

present study indicate that the complete lack of growth of post-settlement oysters in microxia may certainly be due to reduced ingestion rates. My study found no reduction of feeding activity under hypoxic conditions that could account for decreased growth. Exposures to hypoxia of longer durations, however, may significantly reduce ingestion rates and, thus, growth.

In summary, my study demonstrates that oysters have the ability to feed at nearly all stages of settlement and metamorphosis. While hypoxic conditions will affect the feeding activities of only the youngest post-settlement oysters, microxic conditions will affect all post-settlement oysters. This suggests that intrusions of hypoxic water, and especially microxic water, onto oyster beds will limit recruitment into the adult population by decreasing feeding activity and thus survival and growth.

Figure 9. *Crassostrea virginica*. Four phases of metamorphosis (from Baker and Mann, in press) (Chapter 3). (A) Settler. (B) Prodissoconch postlarva. (C) Dissoconch postlarva. (D) Juvenile. *a*, anus; *d*, dissoconch edge; *e*, eyespot; *es*, eyestreak; *f*, foot; *g*, gills; *gr*, gill rudiment; *ilp*, inner labial palps; *m*, mantle edge; *olp*, outer labial palps; *p*, prodissoconch edge; *pf*, pseudofeces; *pam*, posterior adductor muscle; *v*, velum; *vr*, velar remnant. Arrows indicate observed currents.


A
gr
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vr
p
m
C
es
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pam
olp
g
g
f
ilp
p
d
m
pf
D
es
ilp
pam
a
g
p
olp
d
g
m
pf

Figure 10. *Crassostrea virginica*. Ingestion rates, cells h^{-1} individual^{-1}, of post-settlement oysters under normoxic (7.5 mg O_2 l^{-1}), hypoxic (1.9 mg O_2 l^{-1}), and microxic (0.4 mg O_2 l^{-1}) treatments over 6 h periods in relation to duration of exposure. Low oxygen ingestion rates were not determined for the 268 μm oysters. (A) 268 ± 36 μm shell height, S.D., n = 135. (B) 436 ± 60 μm, S.D., n = 135. (C) 469 ± 103 μm, S.D., n = 135. (D) 651 ± 131 μm, S.D., n = 135. (Means ± SD; n = 3)

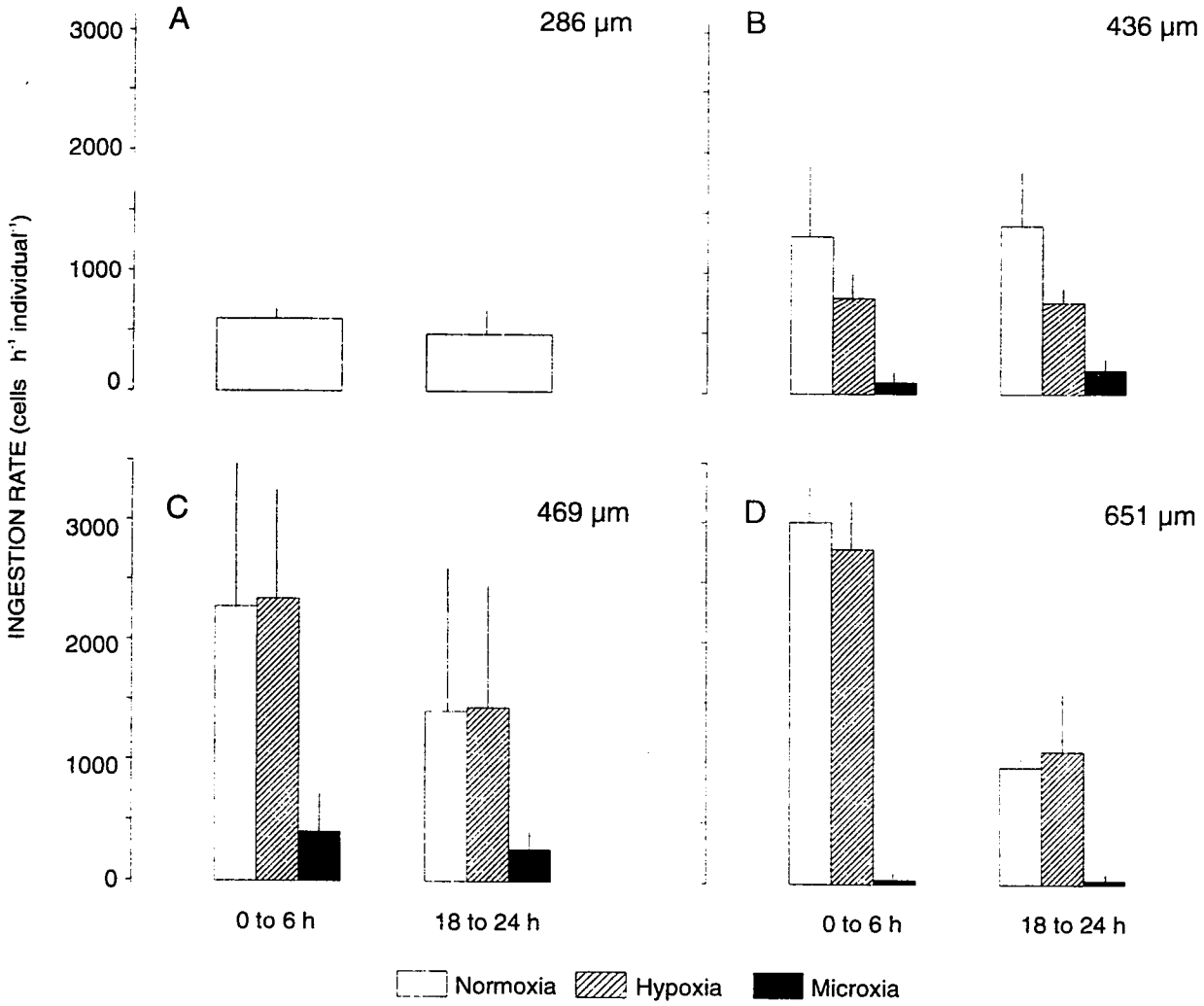
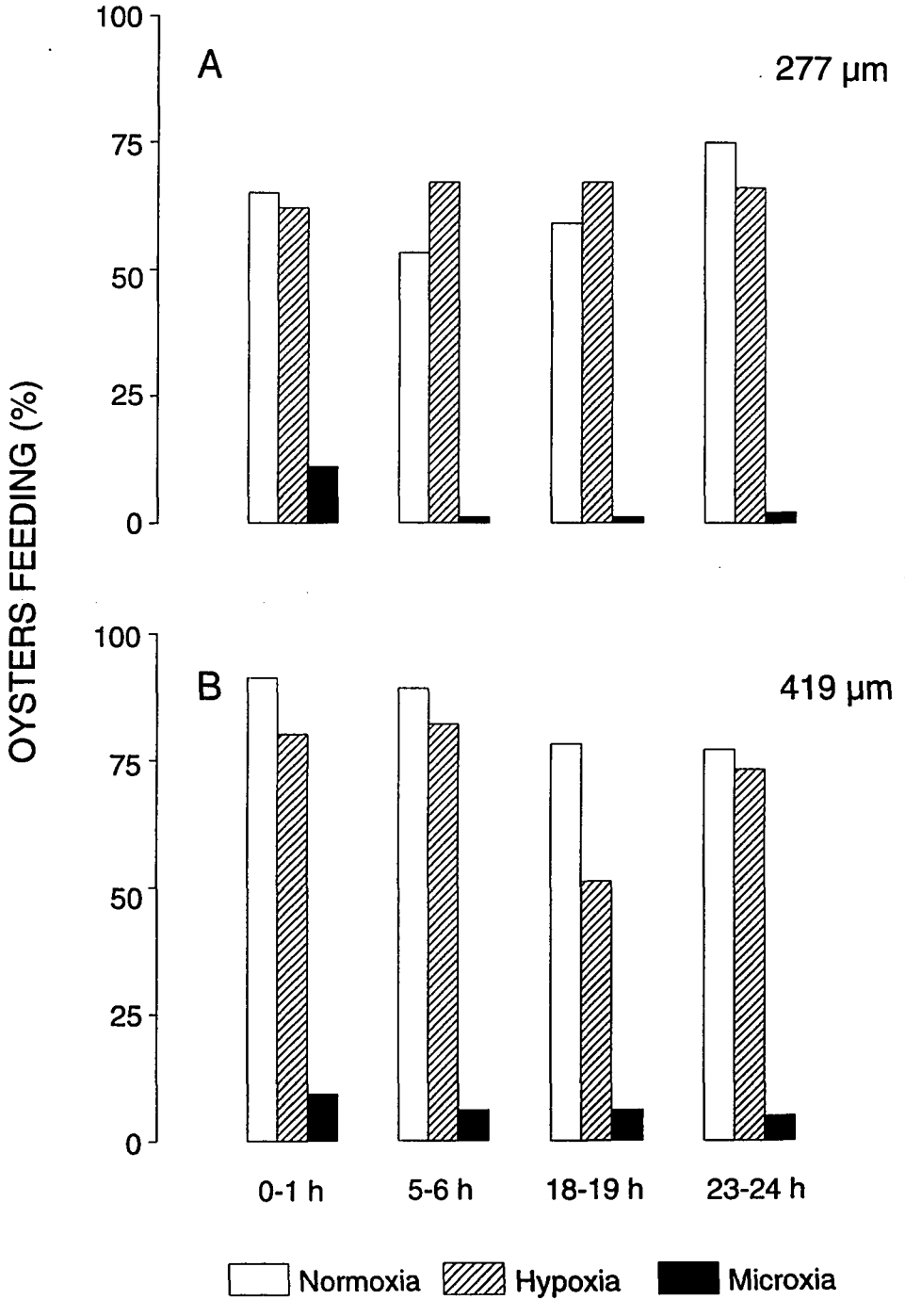

A
286 µm
B
436 µm
C
469 µm
D
651 µm
3000
2000
1000
0
INGESTION RATE (cells h-1 individual-1)
0 to 6 h
18 to 24 h
Normoxia
Hypoxia
Microxia

Figure 11. *Crassostrea virginica*. Effect of normoxic (7.5 mg O_2 l^{-1}), hypoxic (1.9 mg O_2 l^{-1}), and microxic (0.4 mg O_2 l^{-1}) treatments on the proportions of post-settlement oysters feeding over 1 h periods in relation to duration of exposure. (A) 277 ± 31 μm, S.D., n = 240. (B) 419 ± 72 μm, S.D., n = 240.


OYSTERS FEEDING (%)
100
75
50
25
0
A
277 µm
B
419 µm
0-1 h
5-6 h
18-19 h
23-24 h
Normoxia
Hypoxia
Microxia

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Chapter 5

METABOLIC RATES OF METAMORPHOSING OYSTERS, *CRASSOSTREA VIRGINICA*.

Abstract

Microcalorimetry and respirometry were employed to measure the heat dissipation and oxygen uptake rates of metamorphosing oysters *Crassostrea virginica* under normoxic (7.4 mg O_2 l^{-1}), hypoxic (1.5 mg O_2 l^{-1}), and microxic (< 0.07 mg O_2 l^{-1}) treatments. I tested the null hypothesis that there was no effect of oxygen treatment, oyster size/metamorphic phase, or interaction of treatment and size, on weight-specific metabolic rates of metamorphosing oysters. Weight-specific normoxic heat dissipation rates ranged from 78 to 191 μJ h^{-1} μg^{-1} AFDW, generally decreasing with increasing body size (294-601 μm). All sizes reduced their metabolic rates under hypoxia and microxia. Hypoxic rates of heat dissipation were 61-76% of the normoxic rates. In oysters 297 to 387 μm, microxic rates of heat dissipation were 34-49% of the normoxic rates, while 601 μm oysters had microxic rates of only 9%. The ratios of microxic to normoxic rates indicate that metamorphosing oysters are metabolic regulators and switch to conformers as they develop to the juvenile phase. Early post-settlement oysters may have catabolic processes associated with settlement and metamorphosis that cannot be suppressed, resulting in high microxic rates of metabolism. Normoxic rates of oxygen uptake ranged from 54 to 158 pmol O_2 h^{-1} μg^{-1}

AFDW for pediveliger larvae through juveniles (292-465 μm). Weight-specific rates did not decrease with increasing size, however, indicating that there may have been a size-dependent bias in the method of oxygen uptake measurement. My study does demonstrate that there are metabolic changes associated with metamorphosis. Not only does weight-specific metabolism decrease as the oysters grow, but metabolic responses to low oxygen change from relatively oxygen independent to oxygen dependent.

Introduction

Some coastal embayments and estuaries, such as Chesapeake Bay, annually exhibit oxygen depletion as a result of density stratification during the summer months (Officer *et al.*, 1984). The seasonal occurrence of oxygen depletion coincides with the period of settlement of the oyster, *Crassostrea virginica* (Gmelin, 1791). Oxygen depletion is generally restricted to waters below the pycnocline. Wind forcing, however, may tilt the pycnocline, thereby moving hypoxic or microxic deep water into the shallow areas where oyster reefs occur (May, 1973; Carter *et al.*, 1978).

Although several studies have examined the metabolic responses of adult and larval oysters to hypoxia and microxia (Hammen, 1980; Stickle *et al.*, 1989; Widdows, 1989), little is known about the responses of metamorphosing oysters to low oxygen. During the process of metamorphosis, considerable changes in morphology and function take place. Of particular note is the transition from velum to gill as the primary respiratory organ (Cole, 1938; Hickman and Gruffydd, 1969; Fioroni, 1982).

The objective of my study was to measure metabolic rates of metamorphosing oysters under normoxic and low oxygen conditions. Calorimetry was used to measure heat

dissipation rates and respirometry was used to measure oxygen uptake rates. I tested the null hypothesis that there was no effect of oxygen treatment, oyster size/metamorphic phase, or interaction of treatment and size, on weight-specific metabolic rates of post-settlement oysters.

Materials and Methods

Oyster (*Crassostrea virginica*) pediveliger larvae were reared at the Virginia Institute of Marine Science (VIMS) hatchery at Gloucester Point, VA. To produce post-settlement oysters, pediveliger larvae were allowed to settle onto frosted acetate (Mylar) substrate. The substrate was soaked in seawater at least 24 h prior to use to develop a settlement-inducing bacterial coating (Fitt *et al.*, 1990). Larvae which had not settled within 12 h were washed off. To de-attach oysters for experimental purposes, settlers were removed from the substrate with a razor blade. Both larvae and post-settlement oysters, whether attached to substrate or not, were maintained in fingerbowls with filtered (0.45 μm) seawater of 21 ppt. The water was changed every other day and algae food, *Isochrysis galbana*, was added daily at a concentration of approximately 40,000 cells ml^{-1}.

The relationships between weight and shell height (umbo to distal shell margin) were established for larval and unattached post-settlement oysters over the size range used in the experiments. Different sizes of oysters were sieved from the general cultures with appropriate mesh sizes (Nitex) and resuspended in sea water. The shell heights of 20 individuals were measured using a compound microscope and

ocular micrometer. Five to 150 oysters, depending on size, were counted onto pre-washed and pre-ashed glass fiber filters (Gelman A/E) using a microdispensor (Drummond Captrol III). Filters and oysters were washed with distilled water and dried at 80°C for 12 h. The oysters were transferred from the filters to pre-ashed and pre-weighed aluminum pans (12 mm). Pan and oyster weights were determined using a Cahn Model 29 Automatic Microbalance. Pans and oysters were ashed at 500°C for 4 h and immediately placed in a desiccator. After cooling, the pans and oysters were weighed again. Relationships between dry weight, ash-free dry weight, and shell height were based on 12 samples of 3 replicates each. The relationships are described by the following equations:

$$DW = -5.33 - 0.0022SH + 0.000103SH^2 \quad (r^2 = 0.99)$$

$$AFDW = 1.84 - 0.00797SH + 0.000017SH^2 \quad (r^2 = 0.97)$$

where DW is the total dry weight (μg), AFDW is the ash-free dry weight (μg), and SH is shell height (μm).

Calorimetry

Calorimetry was performed at the University of Maine, Orono, Maine. Larvae from the VIMS hatchery were transported on Nitex mesh wrapped in wet paper and packed in an insulated container. Substrates with attached post-settlement oysters, settled 1 month in advance, were packed and transported similarly. Upon arrival, larvae and post-

settlement oysters were placed in seawater (20 ppt) and maintained as described. An algae paste of *Chaetoceras calcitrans* was transported from VIMS and used to feed the animals. To obtain younger post-settlement oysters than those settled in advance, pediveliger larvae were stimulated to settle on substrates with 1% dimethyl sulfoxide (DMSO). Larvae that had not settled within 2 h were washed off.

Total metabolic rates, $\dot{Q}$, of pediveliger larvae and of oysters, 4 h to 24 d post-settlement, were measured by microcalorimetry (LKB 2277 Thermal Activity Monitor, ThermoMetric, Sweden) (see Suurkuusk and Wadsö, 1982, for description). The microcalorimeter was operated in a constant temperature room (25°C ± 1.5°C) and the thermostated water bath was regulated at 25°C ± 0.01°C. Stainless steel ampoules of 5 ml volume were used with 4 ml seawater and an air space. Prior to experiments, oysters were washed with jets of distilled water and seawater from spray bottles. For measurements of heat dissipation by pediveliger larvae, 300 to 800 active individuals were placed in a sample ampoule. For heat measurements of post-settlement oysters, two strips (1 x 3 cm) of substrate with 200 to 900 individuals were placed in a sample ampoule. Oysters were not fed during metabolic measurements.

Heat dissipation of oysters was first determined under treatments of 7.4 mg O_2 l^{-1} (normoxia, 100% of air saturation). After lowering the ampoules into the measuring

cups, equilibrium was typically obtained within 50 min, after which heat dissipation was monitored for approximately 90 min. Some trials included consecutive runs of 1.5 mg O_2 l^{-1} (20% of air saturation) and < 0.07 mg O_2 l^{-1} (< 1% of air saturation) with the same oysters. These treatments, referred to as hypoxia and microxia, respectively, were produced by bubbling a 400 ml water reservoir with appropriate proportions of nitrogen gas and air for at least 90 min. Following a normoxic run, the seawater in both the reference and sample ampoules was replaced several times with hypoxic seawater. The ampoules were sealed under a stream of mixed gas and returned to the measuring cups. The process was repeated with microxic seawater. Following each experimental run, a baseline run was recorded, with the sample ampoule containing seawater but no oysters, at each oxygen concentration used during the experiment.

The pediveliger larvae and post-settlement oysters used in the experiments were preserved in 10% buffered formalin and the shell heights of 30 individuals were measured with a compound microscope and ocular micrometer. Heat dissipation was calculated from the chart traces and corrected for the baseline dissipation. Heat dissipation in nW individual^{-1} was converted to heat dissipation rates, $\dot{Q}$, in μJ h^{-1} individual^{-1} using the conversion factor 1 nW = 3600 μJ h^{-1} (Gnaiger, 1989). Heat dissipation rates were further converted to weight-specific rates, $\dot{q}$, in μJ h^{-1} μg^{-1} AFDW.

Respirometry

Rates of oxygen uptake, $\dot{N}_{O_2}$, by pediveliger larvae and unattached post-settlement larvae, measured as oxygen depletion, were obtained with a Strathkelvin Instruments (SI) microcathode oxygen sensor (1302) held in a SI microrespiration cell with water jacket (RC 200) and connected to a SI oxygen meter (781). The volume of the respiration cell was adjusted to 100 μl. Two microrespiration cell units were connected in series with a water bath regulated at 25°C ± 0.5°C. The oxygen sensor was calibrated daily with air-saturated water (100% of air saturation) and a zero oxygen solution of sodium borate and crystalline sodium sulfite.

Oysters used in the respiration studies were obtained and maintained as described. Oysters of particular sizes and metamorphic phases were picked from the general culture using a microdispensor. The oxygen uptake rates of four metamorphic phases (described in Chapter 3) were determined. Briefly, **settlers** have attached to the substrate but otherwise retain all larval characteristics; both velum and foot are intact and active. A rudiment of the inner demibranch of the left gill is visible. In **prodissoconch postlarvae** the velum has degenerated to a velar remnant and the gill has proliferated. There is no shell growth beyond the prodissoconch. **Dissoconch postlarvae** have completely resorbed the velum but still have a foot. Both the right

and left gills are visible and the labial palps are active. Oysters in this phase have new shell growth at the ventral edge of the prodissoconch. In juveniles metamorphosis is complete; all larval organs are lost. The descending and ascending limbs of the inner demibranch of the left gill are still visible.

The oysters were washed onto Nitex mesh and cleaned with jets of distilled water and seawater from spray bottles. The largest post-settlement oysters required additional cleaning with 1% Chlorox, followed by several seawater rinses, to remove algae from the shells. Five to 100 oysters, depending on size, were counted into the respiration cells containing filtered (0.22 μm) seawater (21 ppt).

Experimental runs included consecutive periods of normoxia, hypoxia, and microxia with the same individuals. Following a normoxic run, the seawater in the respiration cell was equilibrated under a stream of nitrogen gas until the desired hypoxic oxygen concentration was reached. Following the hypoxic run, the process was repeated, depressing the oxygen concentration to the microxic level. Oxygen depletion was monitored for 10 to 30 min, until oxygen concentration changed by about 22%, 6%, and 4% during normoxic, hypoxic, and microxic runs, respectively. This resulted in oxygen uptake being measured over a range of 77% to 98% of air saturation for normoxia, 17% to 23% of air

saturation for hypoxia, and 0.9% to 4.4% of air saturation for microxia. After each experimental run, a baseline was recorded at each oxygen concentration used during the experiment. Shell heights of the oysters used in the experiments were measured and used to calculate DW and AFDW.

Oxygen depletion in the system was calculated from the chart traces and corrected for the baseline oxygen uptake (normoxic and hypoxic runs) or inward diffusion of oxygen (microxic runs). Oxygen uptake in mg O_2 h^{-1} $individual^{-1}$ was converted to pmol O_2 h^{-1} $individual^{-1}$ using the conversion factor 1 mg O_2 h^{-1} = 31.251 x 10^6 μmol O_2 h^{-1} (Gnaiger, 1983). Oxygen uptake was further converted to weight-specific oxygen uptake, $\dot{n}_{O_2}$, in pmol O_2 h^{-1} μg^{-1} AFDW. Experimental oxycaloric equivalents, $\Delta_k H_{O_2}$, were calculated from $\dot{n}_{O_2}$ and $\dot{q}$ for comparison with the generalized $\Delta_k H_{O_2}$ of -450 kJ $(mol\ O_2)^{-1}$ (Gnaiger, 1989).

A two-way analysis of variance was performed to test the null hypotheses that there was no effect of oxygen concentration, oyster size/metamorphic phase, or interaction, on $\dot{n}_{O_2}$. If a null hypothesis was rejected, Tukey's multiple comparison test was performed to determine where differences existed (Minitab 7.2, 1989).

Results

Calorimetry

Heat dissipation rates were determined for *Crassostrea virginica* with shell heights of 294 ± 13 (SD; n = 60), 297 ± 14 (S.D.; n = 240), 337 ± 70 (S.D.; n = 30), 387 ± 107 (S.D.; n = 30), 498 ± 70 (S.D.; n = 30), 529 ± 99 (S.D.; n = 30) and 601 ± 164 μm (S.D.; n = 30) (Fig. 12). These sizes differed in metamorphic phase and gill proliferation. They correspond to larvae (for convenience, hereafter referred to as 294 μm), settlers and prodissoconch postlarvae (297 μm), dissoconch postlarvae (337 and 387 μm) and juveniles (498, 529 and 601 μm). Weight-specific normoxic heat dissipation rates ranged from 78 to 191 $\mu J\ h^{-1}\ \mu g^{-1}$ AFDW, generally decreasing with increasing body size.

Hypoxic and microxic $\dot{q}$ were determined for settlers and prodissoconch postlarvae (297 μm), dissoconch postlarvae (337 μm and 387 μm) and juveniles (601 μm). The oysters reduced their metabolic rates under hypoxia and microxia. The calculated oxygen concentrations at which metabolic rates were 50% of the normoxic rates (0.5 $\dot{q}_{normoxia}$) were 0.4, 1.2, 1.1, and 1.3 mg $O_2\ l^{-1}$ (5.5%, 16%, 15% and 18% of air saturation) for 297, 337, 387 and 601 μm oysters, respectively (Table II).

Hypoxic and microxic rates of heat dissipation were calculated as proportions of the normoxic rates ($\dot{q}_{hypoxia}/\dot{q}_{normoxia}$ and $\dot{q}_{microxia}/\dot{q}_{normoxia}$) (Table II). Hypoxic proportions were relatively similar for all four sizes. In oysters with shell heights of 297, 337, 389 and 601 μm $\dot{q}_{hypoxia}$ was 74, 72, 76 and 61% of $\dot{q}_{normoxia}$, respectively. Proportions of $\dot{q}_{microxia}/\dot{q}_{normoxia}$, however, varied with body size. For oysters 297, 337 and 387 μm in shell height $\dot{q}_{microxia}$ was 45, 34 and 49% of $\dot{q}_{normoxia}$, respectively. In oysters 601 μm, however, $\dot{q}_{microxia}$ was only 9% of $\dot{q}_{normoxia}$.

Respirometry

Rates of oxygen uptake were determined for larvae (292 ± 22 μm, S.D., n = 45), settlers (313 ± 14 μm, S.D., n = 40), prodissoconch postlarvae (300 ± 21 μm, S.D., n = 40), dissoconch postlarvae (399 ± 29 μm, S.D., n = 60) and juvenile oysters (465 ± 29 μm, S.D., n = 40) (Fig. 13). These sizes differed in metamorphic phase and gill proliferation. For convenience, they are sometimes referred to by shell height. Normoxic oxygen rates ranged from 54 to 158 pmol O_2 μg^{-1} h^{-1} AFDW. Oxygen uptake rates varied significantly (two-way ANOVA, P = 0.021 and 0.000) with metamorphic phase and oxygen concentration but there was no significant (two-way ANOVA, P = 0.170) interaction of concentration and phase. The $\dot{n}_{O2(normoxia)}$ of settlers was significantly lower than that of the other metamorphic

phases (Tukey's test, all P values ≤ 0.048) but was not significantly different from settler $\dot{n}_{O2(hypoxia)}$ and $\dot{n}_{O2(microxia)}$ (Tukey's test, both P values > 0.50). Hypoxic and microxic $\dot{n}_{O2}$ did not vary significantly (Tukey's test, $P \leq 0.099$, > 0.50, respectively) with size. For all phases but the settler phase, $\dot{n}_{O2(hypoxia)}$ and $\dot{n}_{O2(microxia)}$ were significantly lower than $\dot{n}_{O2(normoxia)}$ (Tukey's test, $P \leq 0.025$ and < 0.001, respectively) but were not significantly different (Tukey's test, $P \geq 0.051$) from each other.

The calculated oxygen concentrations at which oxygen uptake was 50% of normoxic rates (0.5 $\dot{n}_{O2(normoxia)}$) varied with body size. The calculated concentrations were 0.9, 0.6, 3.3, 3.6, and 3.9 mg O_2 l^{-1} (12, 8, 45, 49, and 53% of air saturation) for larvae, settlers, prodissoconch postlarvae, dissoconch postlarvae and juveniles, respectively.

Calculated experimental oxycaloric equivalents, $\Delta_k H_{O2}$, are shown in Table III. The experimental $\Delta_k H_{O2}$ ranged from -565 to ∞ kJ (mol O_2)$^{-1}$. An anaerobic contribution to the total metabolism is indicated when an experimental $\Delta_k H_{O2}$ is greater than the generalized $\Delta_k H_{O2}$ of -450 kJ (mol O_2)$^{-1}$. The experimental $\Delta_k H_{O2}$ values indicate that there was an anaerobic component to total metabolism in most cases, even under normoxic conditions. The ∞ value for dissoconch postlarvae indicates that there was no oxygen uptake and so metabolism was entirely anaerobic. The experimental $\Delta_k H_{O2}$

reported here must be treated with caution, however, because $\dot{q}$ and $\dot{n}_{O_2}$ were not determined simultaneously.

Discussion

Metabolic rates of metamorphosing oysters have not been previously described. The $\dot{q}$ of *Crassostrea virginica* pediveliger larvae, metamorphosing oysters and small juveniles reported here are comparable to rates published for larvae. Oyster larvae 99, 133, and 300-376 μm in size have $\dot{q}_{normoxia}$ of 132, 99, and 128 $\mu J\ h^{-1}\ \mu g^{-1}$ AFDW (Widdows *et al.*, 1989). The $\dot{n}_{O_2}$ of pediveliger larvae, metamorphosing oysters and small juveniles (reported here) were lower than those noted for larvae. Widdows *et al.* (1989) report $\dot{n}_{O2(normoxia)}$ of 276, 209 and 265 pmol $O_2\ h^{-1}\ \mu g^{-1}$ AFDW for oyster larvae 99, 133 and 300-376 μm in size.

Weight-specific heat dissipation rates of metamorphosing oysters generally decreased with increasing body size while $\dot{n}_{O_2}$ were lowest for settlers but otherwise were not significantly different (Tukey's test, all P values ≤ 0.048) over the size range examined. Rates of metabolism relative to body mass are usually higher in small animals than in larger animals (Hemmingsen, 1960). The fact that this holds true for $\dot{q}$ but not for $\dot{n}_{O_2}$ suggests that there was some size-dependent bias in the method of oxygen uptake measurement. Oxygen uptake in the 100 μl microrespiration cell may be related to the stirring caused by ciliary activity. The smaller metamorphic phases, with less ciliary

activity than larvae and more developed metamorphic phases, may have experienced localized hypoxia resulting in lower $\dot{n}_{O_2}$ than expected.

Due to the uncertainty associated with the $\dot{n}_{O_2}$, no conclusions can be drawn from the rates reported here nor from the calculated $\Delta_k H_{O_2}$. Most of the calculated $\Delta_k H_{O_2}$ far exceed the generalized $\Delta_k H_{O_2}$. Even under normoxic conditions, only the experimental $\Delta_k H_{O_2}$ of juveniles approaches the generalized $\Delta_k H_{O_2}$. Again, this suggests a size related problem in measuring oxygen uptake.

The 0.5 $\dot{q}_{normoxia}$ oxygen concentrations and $\dot{q}_{hypoxia}/\dot{q}_{normoxia}$ of metamorphosing oysters were comparable to those determined for oyster larvae and juveniles (16 mm). Widdows *et al.* (1989) calculated 0.5 $\dot{q}_{normoxia}$ oxygen concentrations of 0.11 to 0.89 mg O_2 l^{-1} (0.27-2.3 kPa pO_2, 1.3-11% of air saturation at 22°C and 12 ppt) for larvae 99 to 300-376 μm. Oyster larvae and juveniles have $\dot{q}_{hypoxia}$ (20% of air saturation) which are 62 to 98% of $\dot{q}_{normoxia}$ (Widdows *et al.*, 1989).

Bivalves generally show one of two responses to microxia. Some maintain a relatively high $\dot{q}_{microxia}/\dot{q}_{normoxia}$ while in others the proportion is small. In oysters, a shift in response to microxia from metabolic regulation to metabolic conformity occurs in the larval stage. One might expect all subsequent life history stages to have a similar response. This does not appear to be the case, however. Metamorphosing oysters also start as metabolic regulators

and switch to conformers as they develop. The smaller metamorphosing oysters (297, 337 and 387 μm), with $\dot{q}_{microxia}/\dot{q}_{normoxia}$ of 45, 34 and 49%, were similar to small oyster larvae in their response to microxia. Prodissoconch (99 μm) and veliconch (133 μm) larvae have $\dot{q}_{microxia}/\dot{q}_{normoxia}$ of 34 and 23% (Widdows *et al.*, 1989). The juveniles in my study (601 μm) had a $\dot{q}_{microxia}/\dot{q}_{normoxia}$ of only 9% which resembles those of pediveliger oyster larvae (300-376 μm) and juveniles (16 mm). Pediveliger oyster larvae and juveniles (16 mm) have $\dot{q}_{microxia}/\dot{q}_{normoxia}$ of only 5 and 3%, respectively (Widdows *et al.*, 1989).

The two types of metabolic responses to microxia, metabolic regulation or metabolic conformity, are usually associated with activity level and microxic tolerance. The relatively high rates of $\dot{q}_{microxia}$ exhibited by prodissoconch and veliconch larvae of the oyster *C. virginica* and prodissoconch larvae of the mussel *Mytilis edulis* correlate with continued activity. These larval stages continue swimming, feeding and growing under low oxygen conditions. Maintenance of swimming ability may allow the larvae to swim away from areas of microxia (Widdows *et al.*, 1989; Wang and Widdows, 1991). Older larvae and juveniles of these two species have low rates of $\dot{q}_{microxia}$ which are accompanied by suppression of feeding and growth (Widdows et *al.*, 1989; Wang and Widdows, 1991; Wang and Widdows, 1993a). The adults of sedentary species of bivalves such as *M. edulis*

and *Abra tenuis* show a similar pattern of suppression of both $\dot{q}$ and activity under low oxygen conditions (Wang and Widdows, 1993a; Wang and Widdows, 1993b).

Metabolic response to low oxygen is also associated with tolerance of such conditions. Animals which maintain $\dot{q}$ and activity in low oxygen, quickly use their energy reserves and, therefore, have short survival times. Prodissoconch and veliconch larvae of oysters and prodissoconch larvae of mussels maintain relatively high $\dot{q}_{microxia}/\dot{q}_{normoxia}$ (23-34%) and have median mortality times (MMT) of only 11 to 15 h (Widdows *et al.*, 1989; Wang and Widdows, 1991). Some adult bivalves also exhibit this pattern of high $\dot{q}_{microxia}$ and low tolerance. For instance, the active bivalve *Mulinia lateralis* maintains a $\dot{q}_{microxia}/\dot{q}_{normoxia}$ of 97% and survives for approximately 5 d (Shumway *et al.*, 1983). Animals which lower their metabolism in response to low oxygen conserve energy and survive longer periods. Older larvae and juveniles of oysters and mussels, with low $\dot{q}_{microxia}/\dot{q}_{normoxia}$ (3-6%), have higher MMTs than their younger counterparts (51-150 h). Adult *Mytilus edulis* suppress their $\dot{q}_{microxia}$ to only 4% of $\dot{q}_{normoxia}$ (Widdows, 1987) and survive microxia for 35 d (Theede *et al.*, 1969).

In metamorphosing bivalves, the relationships between metabolic response to low oxygen, activity and tolerance are unclear. Although the smaller metamorphosing oysters maintained fairly high rates of $\dot{q}_{microxia}$, they do not continue

such activities as growth and development under microxic conditions (Chapter 2, Chapter 3). The relatively high $\dot{q}_{microxia}/\dot{q}_{normoxia}$ of small post-settlement oysters suggests that they would be less tolerant of microxia than pediveliger larvae. Settlers, however, have an MMT of 84 h (Chapter 2) which is longer than that of the larvae (Widdows *et al.*, 1989). Small juveniles, on the other hand, do exhibit the expected reduction in activity associated with their low $\dot{q}_{microxia}/\dot{q}_{normoxia}$. Juveniles (650 μm) reduce rates of ingestion to 1-3% of normoxic rates in response to microxia (Chapter 4). The process of metamorphosis appears to have an unexpected effect on the relationships between $\dot{q}_{microxia}$, activity, and tolerance. Early post-settlement oysters may have catabolic processes associated with settlement and metamorphosis that cannot be suppressed, resulting in high $\dot{q}_{microxia}$.

Simultaneous calorimetric and respirometric experiments are required to more fully understand the metabolism of metamorphosing oysters. My study does demonstrate that there are metabolic changes associated with metamorphosis. Not only does weight-specific metabolism decrease as the oysters grow, but metabolic responses to low oxygen change from relatively oxygen independent to oxygen dependent.

Figure 12. *Crassostrea virginica*. Rates of heat dissipation, $\dot{q}$, under normoxic (7.4 mg O_2 l^{-1}), hypoxic (1.5 mg O_2 l^{-1}) and microxic (< 0.07 mg O_2 l^{-1}) treatments in relation to shell height. Larvae (294 ± 13 μm, S.D., n = 30), settlers and prodissoconch postlarvae (297 ± 14 μm, S.D., n = 240), dissoconch postlarvae (337 ± 70 μm, S.D., n = 30; 387 ± 107 μm, S.D., n = 30), and juveniles (498 ± 70 μm, S.D., n = 30; 529 ± 99 μm, S.D., n = 30; 601 ± 164 μm, S.D., n = 30) . Means + SD. Number of trials listed above bar if other than 1. Na = no data available for hypoxia and microxia.

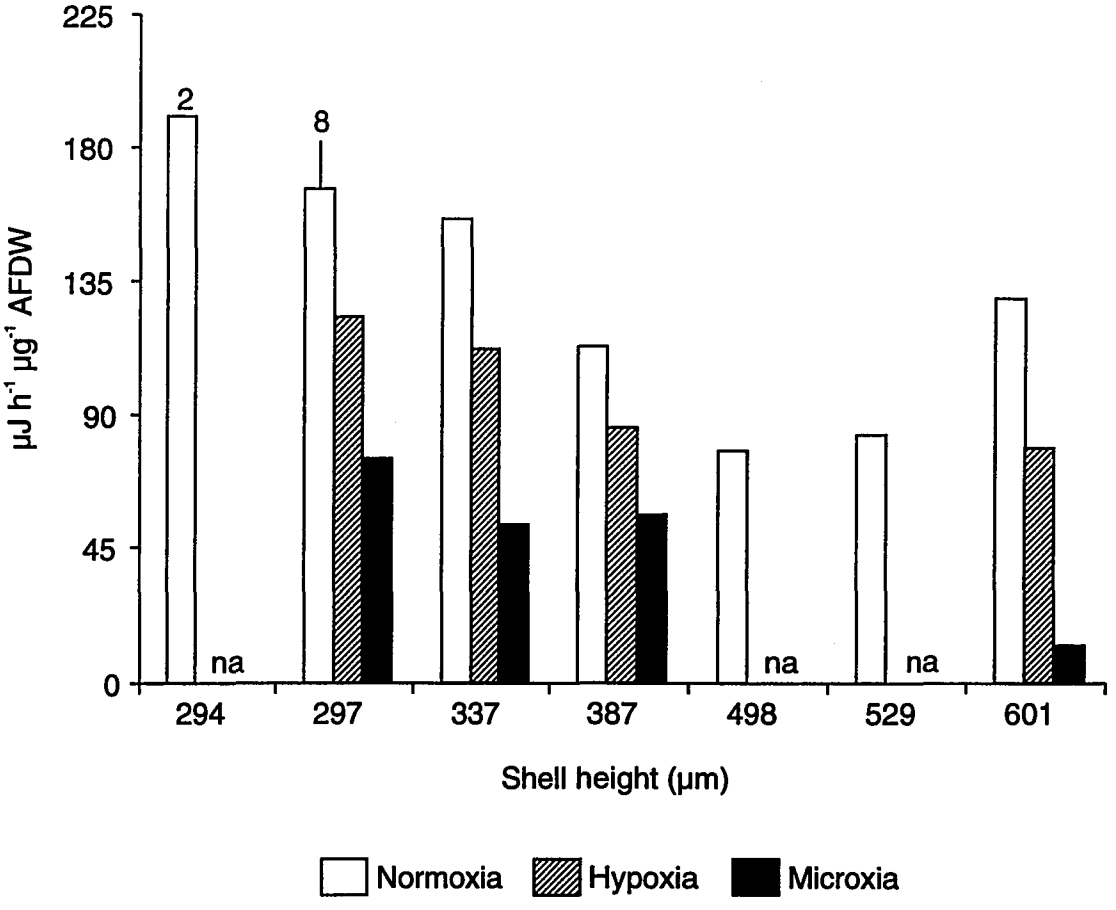
225
180
135
90
45
0
µJ h-1 µg-1 AFDW
2
8
na
na
na
294
297
337
387
498
529
601
Shell height (µm)
Normoxia
Hypoxia
Microxia

Table II. *Crassostrea virginica.* Descriptors of the effects of hypoxia (1.5 mg O_2 l^{-1}) and microxia (< 0.07 mg O_2 l^{-1}) on the weight-specific heat dissipation rates ($\dot{q}$) of metamorphosing oysters.

	Mean shell height (μm)			
	297	337	387	601
0.5 $\dot{q}_{normoxia}$[a] (mg O_2 l^{-1})	0.4	1.2	1.1	1.3
$\dot{q}_{hypoxia}/\dot{q}_{normoxia}$[b]	0.74	0.72	0.76	0.61
$\dot{q}_{microxia}/\dot{q}_{normoxia}$[c]	0.45	0.34	0.49	0.09

[a] Calculated O_2 concentration at which $\dot{q}$ is 50% of the normoxic rate ($\dot{q}_{normoxia}$).
[b] Hypoxic heat dissipation rate ($\dot{q}_{hypoxia}$) as a proportion of $\dot{q}_{normoxia}$.
[c] Microxic heat dissipation rate ($\dot{q}_{microxia}$) as a proportion of $\dot{q}_{normoxia}$.

Figure 13. *Crassostrea virginica.* Oxygen uptake rates under normoxic (7.4 mg O_2 l^{-1}), hypoxic (1.5 mg O_2 l^{-1}) and microxic (< 0.07 mg O_2 l^{-1}) treatments in relation to shell height. Larvae (292 ± 22 μm, S.D., n = 45), settlers (313 ± 14 μm, S.D., n = 40), prodissoconch postlarvae (300 ± 21 μm, S.D., n = 40), dissoconch postlarvae (399 ± 29 μm, S.D., n = 60), and juveniles (465 ± 29 μm, S.D., n = 40). Means + SD. Number of trials listed above bars. Numbers in parentheses indicate number of trials in which no oxygen uptake was detected.

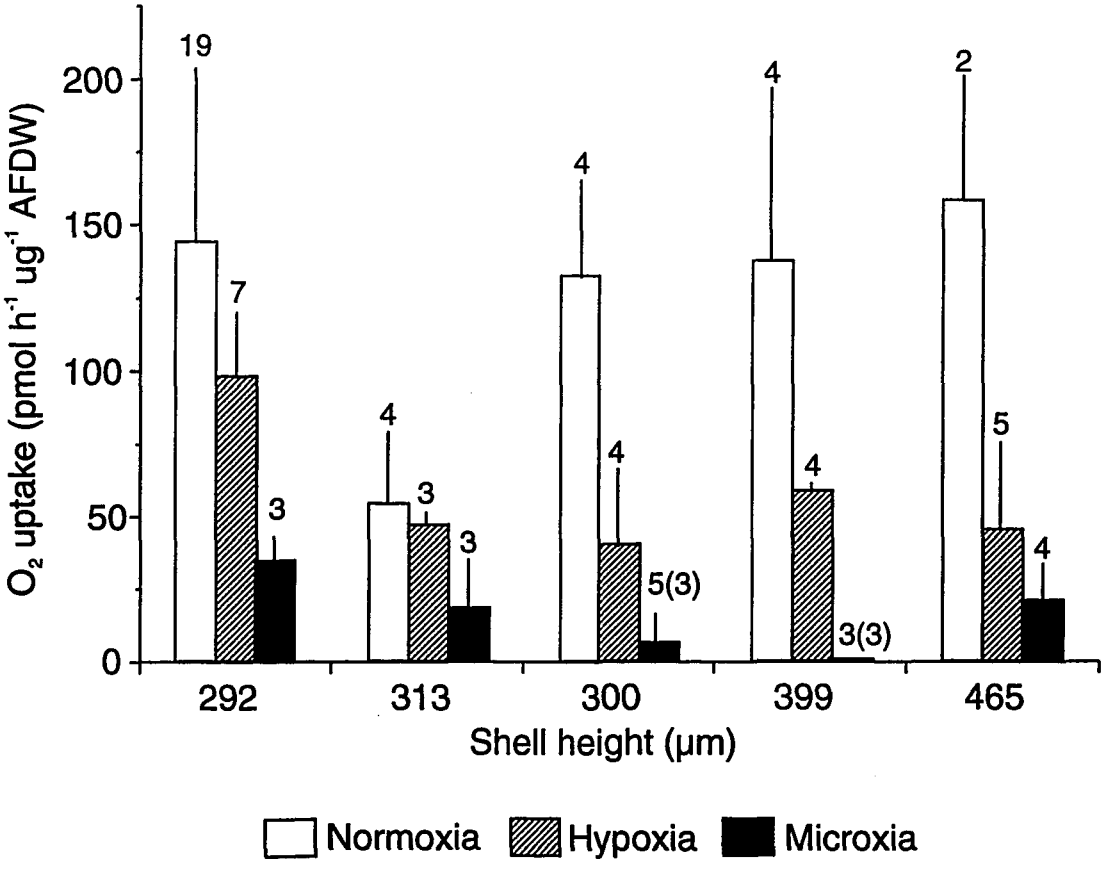
19
7
3
4
3
3
4
4
5(3)
4
4
3(3)
2
5
4
200
150
100
50
0
O_2 uptake (pmol h^{-1} ug^{-1} AFDW)
292
313
300
399
465
Shell height (µm)
Normoxia
Hypoxia
Microxia

Table III. *Crassostrea virginica*. Experimental oxycaloric equivalents ($\Delta_k H_{O_2}$ in kJ (mol O_2)$^{-1}$), calculated from heat dissipation rates ($\dot{q}$) and oxygen uptake rates ($\dot{n}_{O_2}$) under normoxic (7.4 mg O_2 l^{-1}), hypoxic (1.5 mg O_2 l^{-1}) and microxic (< 0.07 mg O_2 l^{-1}) treatments.

Metamorphic phase	Oxygen concentration (mg O_2 l^{-1}) 7.3	1.5	< 0.07
Larvae	-1333	-1268	-2206
Settlers	-3022	-2565	-3667
Prodissoconch postlarvae	-1253	-3128	-12166
Dissoconch postlarvae	-798	-1466	∞
Juveniles	-565	-1822	-2100

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Chapter 6

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary, Conclusions and Recommendations

The oyster *Crassostrea virginica* has historically been a valuable part of the Chesapeake Bay benthic community, but the population has suffered rapid deterioration in recent decades. The decrease in oyster production has been associated with disease, over fishing, low water quality (sedimentation, toxicants, oxygen depletion), and a decline in recruitment. The recruitment period of the oyster coincides with the occurrence of low oxygen events in many of the bays throughout its range (eg. Long Island Sound, Chesapeake Bay, Pamlico Sound).

Previous studies on the effects of low oxygen on bivalves have focused on larval and adult stages and little information is available for the pivotal life history stages of settlement and metamorphosis. The purpose of my research, therefore, was to examine the physiology and behavior of settling and metamorphosing oysters and to investigate the effects of low oxygen stress on metamorphic processes. Specifically, I examined the effects of hypoxia (20% of air saturation) and microxia (< 1% of air saturation) on settlement, survival, growth, morphology, metabolic rates, and feeding.

I began (Chapter 2) by examining the effects of low oxygen on larval settlement success, and post-settlement

growth and survival. I successfully tested the null hypotheses listed in Chapter 1. Larval settlement was reduced significantly in hypoxic treatments, as compared to normoxic treatments, and almost no settlement took place in microxic treatments. At 24 h microxic and normoxic treatment means of larval settlement were significantly different, and at 48 h the microxic treatment mean was significantly different from both the hypoxic and normoxic treatment means. At 72 and 96 h all three treatment means were significantly different from each other.

The regression coefficients of the normoxic and hypoxic treatments were not significantly different; however, the regression elevations were significantly different from each other. In the first 144 h after settlement, post-settlement oysters in hypoxic treatments grew one at one third the rate of those in normoxic treatments, while post-settlement oysters in microxic treatments did not grow at all.

Post-settlement survival was similar in all three treatments for the first 72 h. At 96 h and 120 h the microxic treatment mean was significantly different from both hypoxic and normoxic treatment means. All three treatment means were significantly different from each other at 144 h. Median mortality times of post-settlement oysters in hypoxic and microxic treatments were 131 h and 84 h, respectively.

The results of Chapter 2 indicate that settlement may be an energetically costly activity that oyster pediveliger larvae are unable to complete when in oxygen-limiting environments. The median mortality times indicate that, like larvae and adults, post-settlement oysters are capable of anaerobic metabolism. Chapter 2 demonstrates that hypoxic and microxic conditions have detrimental effects on larval settlement, post-settlement growth, and post-settlement survival.

I became interested in the possibility that low oxygen may have an effect on the morphological changes that take place during metamorphosis. In Chapter 3 I developed a scheme of metamorphic phases that are easily identifiable in live animals using observation and photography. Four phases of metamorphosis were characterized: 'settlers' have attached to the substrate but retain larval characteristics, metamorphosis and degeneration of the velum has begun in 'prodissoconch postlarvae', in 'dissoconch postlarvae' shell growth beyond the prodissoconch has begun but the foot persists and 'juveniles' have lost all larval organs and metamorphosis is complete. These phases were used in examining the metamorphic process during and following continuous and short-term exposures to hypoxia and microxia.

I successfully tested the null hypotheses listed in Chapter 1. The difference between the proportions of juveniles at 120 h in continuous normoxic and hypoxic

treatments was larvae, but were not significantly different from each other. They were, however, both significantly different from the microxic treatments in which no oysters reached the juvenile phase.

I observed no abnormal development, but development was delayed following short exposures (1-3 d) to hypoxia and microxia. However, no significant effect of exposure time on the proportions of juveniles at 14 d post-settlement could be detected. Nor was there interaction of exposure time and oxygen treatment. There was, however, a significant difference between the proportions of juveniles on Day 14 in the normoxic and microxic treatments.

Approximately 50% of the control oysters died within the 14 d period following settlement. Short-term exposures (1-3 d) to hypoxia had little affect on the median mortality time or final total mortality, compared to controls. Microxic treatments longer than one day, however, increased mortality.

The slopes of the nine oxygen treatment/exposure time combinations were not significantly different from each other. This indicated that short-term exposures to low oxygen did not have permanent affects on post-settlement growth rates. There were significant differences in the elevations of the lines, indicating that oysters exposed to microxic treatments had slower growth rates during the exposure period.

The results of Chapter 3 suggest that the 14 d period following settlement is especially critical to recruitment. Low oxygen conditions, especially microxic conditions longer than 24 h, increase mortality and have detrimental effects on the development and growth of post-settlement oysters.

In Chapter 4 I examined the feeding ability of oyster larvae during settlement behavior and metamorphosis, and the effects of hypoxia and microxia on ingestion rates of post-settlement oysters. Feeding occurred during the searching and crawling stages of settlement behavior, but not during cementation. In newly settled larvae, 16% engaged in velar feeding. All later metamorphic phases ingested particles, although the mode of particle capture was unclear.

Oxygen treatment had a significant effect on the ingestion rates of the three sizes of oysters in which it was measured. For oysters of 436 μm shell height, ingestion rates were significantly different in all three oxygen treatments. Exposure time and the interaction of oxygen treatment and time were not significant for this size oyster.

The ingestion rates of oysters with mean shell heights of 469 μm were not significantly different in normoxic and hypoxic treatments. Microxic ingestion rates, however, were significantly lower than both normoxic and hypoxic rates. Microxic rates of ingestion were 18% of normoxic rates.

In the largest size post-settlement oysters examined (651 μm), normoxic and hypoxic ingestion rates were not significantly different from each other and both were significantly greater than microxic ingestion rates. Unlike the previous size classes, the effect of exposure time and the interaction of exposure time and oxygen treatment were significant. Both normoxic and hypoxic ingestion rates decreased significantly over the 24 h exposure period.

My feeding studies demonstrated that oysters have the ability to feed at nearly all stages of settlement and metamorphosis. While hypoxic conditions affect the feeding activities of only the youngest post-settlement oysters, microxic conditions will affect all post-settlement oysters.

The final chapter (Chapter 5), examines the metabolic rates of metamorphosing oysters in normoxic and low oxygen conditions. I used microcalorimetry to measure heat dissipation and respirometry to measure oxygen uptake. I was unable to test any hypotheses about the heat dissipation data because of a lack of replicates. Weight-specific normoxic heat dissipation rates decreased with increasing body size. All sizes reduced their metabolic rates under hypoxia and microxia. Hypoxic rates of heat dissipation were 61-76% of normoxic rates. In the smaller sizes of post-settlement oysters, microxic rates of heat dissipation were 34-49% of the normoxic rates, while 601 μm oysters had microxic rates of only 9%. The ratios of microxic to

normoxic rates indicate that metamorphosing oysters are metabolic regulators and switch to conformers as they develop to the juvenile form.

Weight-specific rates of oxygen uptake did not decrease with size, indicating that there may have been a size-dependent bias in the method of oxygen uptake measurement. I tested the null hypotheses listed in Chapter 1 but they may not be correct. Oxygen uptake rates varied significantly with metamorphic phase and oxygen concentration but there was no significant interaction of concentration and phase.

Chapter 5 demonstrates that there are metabolic changes associated with metamorphosis. Not only does weight-specific metabolism decrease as the oysters grow, but metabolic responses to low oxygen change from relatively oxygen independent to oxygen dependent.

A critique of my experimental and statistical design, based on Hurlbert (1984), is in order. First, "experimental units", individual oyster larvae or substrate units, were not randomly assigned to treatments. My assignment system could perhaps be termed "haphazard". Second, I did not determine homogeneity of the oyster larvae or substrate units which I assigned to treatments. Nor were my treatments themselves randomized in space or "interspersed". I always had normoxic treatments on one end of the lab bench, hypoxic treatments next, and microxic treatments at

the other end. Although this represented a small area, it is possible that differences between location existed. Differences between locations may have become greater during the experiments. These changes could have involved lighting, temperature, etc. I also tended to measure or observe the normoxic treatments first, hypoxic treatments next, and microxic treatments last, on a given day. This may have introduced bias on my part if I became less careful as the day went on.

According to Hurlbert (1984), replications which are temporally segregated are not independent. In Chapters 2, 3, and 5, my replicates were segregated over time, sometimes by several months. This is further confounded by the fact that temporally segregated replicates used different cohorts of larvae. Gallager et al. (1986) observed a positive correlation between egg quality, measured as lipid content, and the proportion of *C. virginica* and *Mercenaria mercenaria* larvae completing metamorphosis. Borsa et al. (1992) reported a positive relationship between heterozygosity and survival of microxic stress in the bivalve *Ruditapes decussatus*. If egg quality and/or heterozygosity of the several cohorts of larvae that I used differed, these factors, would have contributed to the degree of heterogeneity between temporally segregated replicates. When such replications are used to test for treatment effects, pseudoreplication occurs.

Sacrificial pseudoreplication occurs when true replication of treatments existed but the data were pooled prior to statistical analysis. In this case variance among treatment replications exists in the original data but is thrown away when the data is pooled. I committed this type of pseudoreplication in Chapters 2 and 3 when I pooled growth data.

In summary, my dissertation demonstrates that settlement success, survival, growth, morphology and feeding of metamorphosing oysters respond similarly to low oxygen. It raises a question, however, about the relationship between these parameters and metabolism. My study does indicate that there are metabolic changes associated with metamorphosis. It suggests that oyster distribution may be influenced by low oxygen, especially in those areas that experience prolonged (24-48 h) or severe (microxic) pycnocline tilt events. Pycnocline tilt events may control recruitment into the adult population directly, because of larval settlement failure and post-settlement mortality, and indirectly, because of a reduction in feeding, development rate, and growth of post-settlement oysters.

Based on the results of my dissertation and what is generally known about the effects of low oxygen on bivalves, I would like to offer some recommendations concerning the oyster fishery in Chesapeake Bay. Research should be

performed to compare the susceptibility of the native oyster and the Japanese oyster, *Crassostrea gigas*, to low oxygen. If the Japanese oyster is resistant to both hypoxia and disease, introduction to the bay could be considered. Aquaculture of oysters should be promoted. Oysters could be grown in mechanically aerated areas or in off-bottom racks where oxygen depletion is not a problem. "Water wings", developed by the physical oceanographers to break up stratification, could be tethered around especially productive oyster beds. Breaking up stratification would keep the area well aerated. Dissolved oxygen should be monitored in potential sanctuary and repletion areas. If oxygen is low, other areas should be considered. Once a sanctuary or repletion area has been established, oxygen should continue to be monitored. Homeowners, industry, and farmers around the Chesapeake should be encouraged to decrease their nutrient input to the bay. Decreased nutrients in the bay will decrease the eutrophication that exacerbates hypoxia and microxia.

To conclude, my dissertation research builds upon previous observations of the effects of low oxygen conditions on the physiology of larval and adult bivalves. It fills a stage-specific gap in our knowledge of the oyster, *C. virginica*. Understanding the effects of physical conditions such as hypoxia and microxia, which may influence settlement and metamorphic success, is important in a

practical sense in designing future measures to revitalize the rapidly deteriorating Chesapeake Bay oyster fishery. On a broader perspective, my research contributes to understanding the physical factors limiting settlement and recruitment to the benthos of Chesapeake Bay.

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Appendix A

UNIT CONVERSION FACTORS

Unit Conversion Factors

Table IV. Conversion factors for units of amount of oxygen.*

	= µmol	mg	ml
1 µmol	= 1	0.03199	0.022392
1 mg	= 31.251	1	0.69978
1 ml	= 44.659	1.4290	1

* Adapted from **Forstner, H., and E. Gnaiger. 1983.** Appendix A: Calculation of equilibrium oxygen concentration. Pp. 321-333 in *Polarographic oxygen sensors: Aquatic and Physiological Applications*, E. Gnaiger and H. Forstner, eds. Springer-Verlag, Berlin.

Table V. Conversion factors for units of pressure.*

	= kPa	mm Hg	Torr	Atm
kPa	= 1	7.5006	7.5006	0.0098692
mm Hg	= 0.13332	1	1	0.0013158
Torr	= 0.13332	1	1	0.0013158
Atm	= 101.325	760	760	1

* Adapted from **Forstner, H., and E. Gnaiger. 1983.** Appendix A: Calculation of equilibrium oxygen concentration. Pp. 321-333 in *Polarographic Oxygen Sensors: Aquatic and Physiological Applications*, E. Gnaiger and H. Forstner, eds. Springer-Verlag, Berlin.

Table VI. Conversion factors for units of oxygen consumption and heat dissipation on the basis of a generalized oxycaloric equivalent, $\Delta_K H_{O_2}$ = -450 kJ (mol O_2)$^{-1}$.*

	$\dot{N}_{O_2}$ = μmol O_2 h^{-1}	nmol O_2 s^{-1}	$\dot{Q}$ mW	J h^{-1}
1 μmol O_2 h^{-1}	= 1	0.27778	0.1250	0.450
1 nmol O_2 s^{-1}	= 3.600	1	0.450	1.620
1 mg O_2 h^{-1}	= 31.251	8.6809	3.906	14.06
1 cm^3 O_2 h^{-1}	= 44.615	12.393	5.577	20.08
1 mW	= 8.000	2.222	1	3.600
1 J h^{-1}	= 2.222	0.6173	0.27778	1
1 cal h^{-1}	= 9.304	2.584	1.1630	4.187

* Adapted from **Gnaiger, E. 1983.** Appendix C: Calculation of energetic and biochemical equivalents of respiratory oxygen consumption. Pp. 337-345 in *Polarographic oxygen Sensors: Aquatic and Physiological Applications*, E. Gnaiger and H. Forstner, eds. Springer-Verlag, Berlin.

Appendix B

SYMBOLS, DESCRIPTIONS, AND UNITS

Symbols, Descriptions, and Units

Table VII. List of symbols, descriptions, and units.*

Symbol	Description	Units
cO_2	amount-of-substance concentration	μmol dm^{-3}
C^*	unit standard concentration of oxygen	μmol dm^{-3}
$\Delta_k H_{O2}$	caloric equilvalent of oxygen consumption	kJ mol^{-1}
N_{O2}	amount of oxygen	mol
$\dot{N}_{O2}$	oxygen uptake rate	μmol h^{-1}
$\dot{n}_{O2}$	weight specific rate of oxygen consumption	μmol h^{-1} g^{-1}
pO_2	partial pressure of oxygen	kPa
POS	polarographic oxygen sensor	
Q	heat	J
$\dot{Q}$	rate of heat dissipation	μJ h^{-1} or mW
$\dot{q}$	weight specific rate of heat dissipation	μJ h^{-1} g^{-1} or mW g^{-1}
S_s	solubility of oxygen in the sample medium	μmol dm^{-3} kPa^{-1}
T	absolute temperature	K
W	weight	g
θ	Celsius temperature	°C

* Adapted from **Gnaiger, E. 1983.** Appendix E: Symbols and units: Toward standardization. Pp. 352-358 in *Polarographic Oxygen Sensors: Aquatic and Physiological Applications*, E. Gnaiger and H. Forstner, eds. Springer-Verlag, Berlin.

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