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Effects of periodic hypoxia on mortality, feeding and predation in an estuarine epifaunal community ☆

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Abstract

The York River Estuary, a tributary of the Chesapeake Bay, USA, experiences periodic low oxygen stress (hypoxia), yet epifaunal species form dense communities there. We studied hypoxia tolerance of common epifaunal species in the York River by exposing sessile and mobile epifauna to high and low oxygen concentrations in laboratory aquaria. Mortality in hypoxia varied among species, ranging from 0% to 100%, with trends of decreased tolerance by mobile species relative to sessile species. While most species tested experienced some mortality after being exposed to hypoxia (at 1 mg O_2/l or 0.5 mg O_2/l) for 5 days, many species had a median lethal time (LT_{50}) in hypoxia greater than 1 week (3 of 6 species at 1 mg O_2/l and 6 of 14 species at 0.5 mg O_2/l), the maximum duration of typical hypoxic episodes in the York River, suggesting that hypoxia may cause little mortality for some species in this system. However, hypoxia had sub-lethal effects on behavior in all species tested. Epifaunal animals responded to hypoxia with behaviors that moved them higher in the water column or by entering resting states until hypoxia passed. Feeding and predation by a variety of taxa (the hydroid *Obelia bicuspidata*, the mud crab *Neopanope sayi*, juvenile blue crabs *Callinectes sapidus*, the flatworm *Stylochus ellipticus*, and the nudibranch *Doridella leucolena*) decreased during hypoxia, despite varying mortality responses to low oxygen stress, suggesting that short hypoxic episodes may create predation refuges for prey species. At least one highly tolerant species (*O. bicuspidata*) showed substantially decreased growth in hypoxia. Although relatively high tolerance of hypoxia by many estuarine epifaunal species limits serious disturbance during brief hypoxic episodes, hypoxia's greatest impact on York River

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epifaunal communities might be through its indirect effects on behavior and predation.

Keywords: Epifauna; Estuaries; Hypoxia; Predation

1. Introduction

The tolerance of stress by a species can influence its distribution and abundance (Sousa, 1979; Grossman et al., 1998) by changing the likelihood of disturbance (Sousa, 1984; Connell et al., 1997), the rate of population growth (Huston, 1979, 1994) and the outcome of interactions with other species (Menge, 1978; Witman and Grange, 1998). In some aquatic communities, benthic invertebrates experience stress from low oxygen, termed hypoxia. Dissolved oxygen concentrations in the water column of 2 mg O_2/l or lower often have deleterious effects on animals and are considered hypoxic (Tyson and Pearson, 1991). Hypoxia occurs worldwide in lakes, estuaries and coastal areas (Diaz and Rosenberg, 1995). In many systems, anthropogenic eutrophication is increasing the frequency and duration of hypoxia by increasing microbial respiration (Diaz and Rosenberg, 1995).

The effects of hypoxia on biotic communities depend in part on its severity (how low dissolved oxygen concentrations fall) and duration. In extreme cases, where hypoxia lasts for many weeks or approaches anoxia (a complete lack of dissolved oxygen), hypoxia can cause emigration of mobile animals (Diaz and Rosenberg, 1995) and mass mortality of sessile animals (Jørgensen, 1980; Stachowitsch, 1984). When oxygen conditions are milder (dissolved oxygen concentrations remain well above anoxic levels) or hypoxic episodes are brief, hypoxia can change species composition (Josefson and Widbom, 1988; Llanso, 1992) and reduce benthic biomass (Dauer et al., 1992).

Tolerance to hypoxia varies among taxonomic groups and habitats. Polychaetes, bivalves, platyhelminths and cnidarians are relatively tolerant, while crustaceans and vertebrates have relatively low hypoxia tolerance (Mangum and van Winkle, 1973; Diaz and Rosenberg, 1995). Animals from habitats that often experience low oxygen may be more tolerant than animals less likely to encounter hypoxia (Theede et al., 1969; Sassaman and Mangum, 1972; Vistisen and Vismann, 1997). Some studies suggest that infaunal species, which live within the sediment, tolerate hypoxic stress better than epifaunal species, which live on the sediment surface (Hagerman, 1998). For example, authors have compared the hypoxia tolerance of infaunal and epifaunal species of anemones (Sassaman and Mangum, 1972), brittle stars (Vistisen and Vismann, 1997), snails (McMahon and Russell-Hunter, 1978) and polychaetes (Theede et al., 1973) and found that infaunal species were more tolerant in each case. However, some epifaunal groups are abundant in areas with low oxygen, suggesting that these species may be highly tolerant of hypoxia (Tunnicliffe, 1981; Sagasti et al., 2000). The tolerance of hypoxia by many epifaunal groups (such as hydroids and bryozoans) is unknown.

In addition to killing animals directly, hypoxia can change behavior (Hagerman, 1998), feeding rates, growth (Forbes and Lopez, 1990) and the outcome of species

interactions, including predation (Breitburg et al., 1994; Nestlerode and Diaz, 1998; Legeay and Massabuau, 2000). Many species respond to hypoxia with behaviors that help them stretch higher into the water column where oxygen concentrations are generally higher (Hagerman, 1998; Diaz and Rosenberg, 1995). Hypoxia depresses feeding in some species (Sobral and Widdows, 1997; Rosas et al., 1998) while increasing it in others (Breitburg et al., 1994). The relative tolerance of predators vs. prey may determine whether predation rates increase or decrease during hypoxia (Breitburg et al., 1994). For example, predation on larval fish by jellyfish can increase during hypoxic episodes, because jellyfish tolerate hypoxia, but larval fish are unable to evade predators in low oxygen (Breitburg et al., 1994). Concurrently, predation on larval fish by adult fish decreases, because the adult fish are intolerant of hypoxia (Breitburg et al., 1994). Some predators switch prey species as oxygen falls, preferring to exploit the most intolerant prey (Sandberg, 1994). In areas with fluctuating oxygen levels, mobile predators can temporarily leave hypoxic areas, then return when oxygen rises to take advantage of infaunal invertebrates that surface during low-oxygen events (Pihl et al., 1992; Rahel and Nutzman, 1994; Nestlerode and Diaz, 1998).

Epifaunal animals such as barnacles, anemones, hydroids, and tunicates are abundant in the York River Estuary, Chesapeake Bay, USA (Schaffner et al., 2001) despite frequent hypoxia (Kuo and Neilson, 1987). Epifaunal species in the Chesapeake Bay have highly seasonal distributions and are most abundant in the summer (Abbe, 1987; Sagasti et al., 2000), which is also when hypoxia occurs (Kuo and Neilson, 1987). In the York River, oxygen concentrations follow relatively predictable cycles, with hypoxia occurring in June–September during neap tides and disappearing during spring tides (Haas, 1977). Hypoxic episodes last from several days up to a week, with typical duration of 5 days (Kuo et al., 1993). Epifaunal communities in the York River have similar species composition in deep areas where hypoxia is common and in shallow areas where hypoxia does not occur (Sagasti et al., 2000). In addition, species abundance is similar throughout the lower York River (Sagasti et al., 2000), even though some areas experience lower oxygen concentrations than others (Sisson et al., 1991; Sagasti et al., 2000). This suggests that epifaunal species characteristic of the York River may be resistant to periodic hypoxia.

Species interactions such as predation may be important for structuring epifaunal communities in the Chesapeake Bay (Branscomb, 1976; Marsh, 1976; Rheinhardt and Mann, 1990), and hypoxia could change these interactions (Breitburg et al., 1994). Major predators on epifaunal species in the Chesapeake Bay include fish, crabs (Rheinhardt and Mann, 1990), flatworms (Branscomb, 1976), snails and nudibranchs (Marsh, 1976). Large crabs and fish leave low-oxygen areas during hypoxic episodes, but may return after these episodes to feed on stressed prey items (Pihl et al., 1992; Nestlerode and Diaz, 1998). Small predators such as mud crabs and juvenile blue crabs belong to a relatively intolerant phylogenetic group (i.e. crustaceans, Gerhardt and Baden, 1998) and may be too small to escape hypoxic areas; therefore, their predation rates might decrease during hypoxia. Other small predators such as flatworms and snails belong to relatively tolerant phylogenetic groups (McMahon and Russell-Hunter, 1978; Armonies, 1986) and might increase feeding rates if hypoxia makes their prey more vulnerable.

This study examined the hypoxia tolerance in epifaunal species of the York River Estuary. We considered the effects of hypoxia on mortality, behavior, feeding rates and predation. For the most abundant epifaunal species in the York River Estuary, we calculated the median lethal time (LT_{50}) at two oxygen concentrations (1 and 0.5 mg O_2/l) common in the York River during hypoxic episodes to determine whether tolerance varies among species and whether short hypoxic episodes at these oxygen concentrations could cause widespread mortality. We also investigated the effects of hypoxia on feeding by a highly tolerant sessile species and on predation by the most abundant small, mobile predators in this system to determine if hypoxia changes feeding rates and if prey might use hypoxic areas as predation refuges. Finally, we relate the tolerance of individual species to their abundance in hypoxic areas to examine whether hypoxia could influence the distribution of epifaunal species in this system.

2. Methods

2.1. Collection of epifauna

We tested the hypoxia tolerance of dominant epifaunal species in the York River, Chesapeake Bay, USA during the summer when hypoxia occurs (Kuo and Neilson, 1987). We collected eight of the nine most common sessile species, as determined during a previous study in this system (Sagasti et al., 2000). We also collected seven mobile species, six of which were abundant during the previous study (Sagasti et al., 2000) and one (juvenile blue crab *Callinectes sapidus*) which has been found by others to be an important predator in Chesapeake Bay epifaunal communities (Rheinhardt and Mann, 1990). Sessile species were collected by submersing 10×10 cm PVC panels or 2.6×7.6 cm glass microscope slides from the Virginia Institute of Marine Science (VIMS) pier in the York River at a depth of 1–2 m and allowing epifaunal animals to colonize. We collected species after panels or slides had been immersed less than 3 weeks to ensure that populations were young and actively growing. The tube-building polychaete *Sabellaria vulgaris* was collected on empty shells of the clam *Mercenaria mercenaria* under the VIMS pier.

Epifaunal species in the York River have highly seasonal recruitment periods (Sagasti et al., in preparation), so we repeated the collection process several times each summer in 1997–1999 to maximize the diversity of collected species. Although the species tested in this study are all simultaneously present in the York River epifaunal community during the late summer peak in hypoxia, the abundance of each species varies throughout the summer (Sagasti et al., 2000). Each species was tested when it was most abundant (a few weeks after the peak recruitment period). Consequently, species were tested at different times of the summer, and it is possible that this may have influenced the results. For example, the tolerance of some species to low oxygen conditions may change throughout the summer season. Thus, the results of this study allow us to compare the relative tolerance of different species during the time of the summer when each is most abundant, but not necessarily when each is most tolerant of low oxygen.

On each substratum, we removed all but one species. For most species, we left only one animal or colony on each substratum, but for a few species we left multiple animals on each substratum (Table 1), ensuring that individuals did not touch each other. It was difficult to distinguish among colonies of the hydroid *Obelia bicuspidata*, so all animals were removed except for a 1-cm^2 area of hydroid (Table 1). The polychaete *Polydora cornuta* builds U-shaped mud tubes that are entwined together, making it difficult to isolate a single individual, so we left intact a 1-cm^2 area covered with *P. cornuta* tubes (Table 1).

Mobile species were collected by hand from the VIMS pier, except for juvenile blue crabs (*C. sapidus*) which were collected with dip nets in 1–2 m deep eelgrass beds off Goodwin Island (York River, approximately 7 km from VIMS). Mobile animals were placed in one of two cage types, termed "large cages" and "small cages" (Fig. 1). We kept blue crabs and mud crabs (*N. sayi*) in $11 \times 11 \times 7$ cm cages with 1 mm mesh plastic window screening on five sides and a solid plastic back on the sixth side (large cages). Smaller animals were kept in finger bowls (5.5 cm diameter, 2.3 cm height) covered on top by 125 μm nytex mesh (small cages). In one experiment (15–20 August 1999), we put mud crabs in small cages with 1 mm mesh rather than 125 μm mesh. We held animals in aquaria with flow-through York River water for 1–3 days before using them in experiments.

2.2. Experimental design

In the summers of 1997–1999, we conducted a series of laboratory experiments exposing epifaunal species to high and low oxygen conditions (Table 1). Due to logistical constraints, up to four species shared aquaria in some experiments. However, strong inter-species effects appear unlikely because different species had no physical contact, they shared a water volume much larger than their body volumes, they were fed in excess, and all dead animals were removed immediately.

For each species, we randomly assigned one substratum or cage containing animals to randomly interspersed control (high oxygen) and experimental (low oxygen) 40 l aquaria. Each treatment (high and low oxygen) had 5 (7–12 June 1997), 8 (all remaining experiments in 1997) or 10 (1998–1999) replicate aquaria. Within each aquarium, substrata with sessile epifauna were hung along the sides of aquaria, with animals facing towards the middle of each aquarium (Fig. 1). Large cages were placed with the solid side against the sides of aquaria, and mesh sides facing towards the middle (Fig. 1). Small cages were placed on the bottom of aquaria with the mesh on top (Fig. 1).

Each aquarium was filled with sea water pumped from the VIMS pier and passed through two sand filters to remove animals and debris. Salinity in the York River near VIMS changes predictably each year (Sagasti et al., 2000), rising steadily from spring until fall. Therefore, experiments conducted in June–July each year had salinities of 16–18 ppt, and experiments in August or September had salinities of up to 23 ppt. Experiments were conducted at room temperature (20–26°C), similar to the temperature range in the York River during summer (Sagasti et al., 2000). Aquaria were kept in the dark to mimic deep areas of the York River where hypoxia occurs (although the specimens used in these experiments were collected near the surface, all the species

Table 1
Experimental details showing for each experiment the species tested (number of individuals per replicate or area covered by species), their diet, and mortality criteria

Species	Diet	Mortality criterion
7–12 June 1997		
Neopanope sayi (1)	worm (*Nereis succinea*)	no response after several hours of high oxygen
23–28 June 1997		
N. sayi (1)	barnacle (*Balanus improvisus*)	no response after several hours of high oxygen
Stylochus ellipticus (2)	barnacle (*B. improvisus*)	releases substrate; disintegrates
Mitrella lunata (1)	none	no response after several hours of high oxygen
19–24 August 1997		
Diadumene leucolena (5)	filter feeding block	no response after several hours of high oxygen
Hydroides dianthus (1)	filter feeding block	no response after several hours of high oxygen
24–29 August 1997		
M. lunata (2)	none	no response after several hours of high oxygen
Doridella obscura (1)	bryozoan (*Membranipora tenuis*)	releases substrate; disintegrates
Cratena kaoruae (2)	hydroid (*O. bicuspidata*)	releases substrate; disintegrates
7–11 June 1998		
Molgula manhattensis (1)	algae paste, filter feeding block	no response after several hours of high oxygen
Mem. tenuis (1)	algae paste, filter feeding block	zooid turns black
P. cornuta (1 cm^2)	algae paste, filter feeding block	out of tube; disintegrates
19–25 July 1998		
C. sapidus (1)	barnacle (*B. improvisus*)	no response after several hours of high oxygen
M. lunata (4)	none	no response after several hours of high oxygen
D. obscura (3)	bryozoan (*Mem. tenuis*)	releases substrate; disintegrates
11–16 August 1998		
H. dianthus (1)	algae paste, filter feeding block	no response after several hours of high oxygen
Diad. leucolena (1)	algae paste, filter feeding block	no response after several hours of high oxygen
21–26 June 1999		
B. improvisus (1)	algae paste, liquid filter feeder food	turns black, disintegrates
Conopeum tenuissium (1)	algae paste, liquid filter feeder food	zooid turns black
Mem. tenuis (1)	algae paste, liquid filter feeder food	zooid turns black
Ner. succinea (1)	none	no response after several hours of high oxygen
15–20 August 1999		
S. vulgaris (1)	algae paste, liquid filter feeder food	no response after several hours of high oxygen
Ner. succinea (1)	none	no response after several hours of high oxygen
N. sayi (1)	barnacle (*B. improvisus*)	no response after several hours of high oxygen
O. bicuspidata (1 cm^2)	*Artemia* sp., live plankton	no growth after days in high oxygen

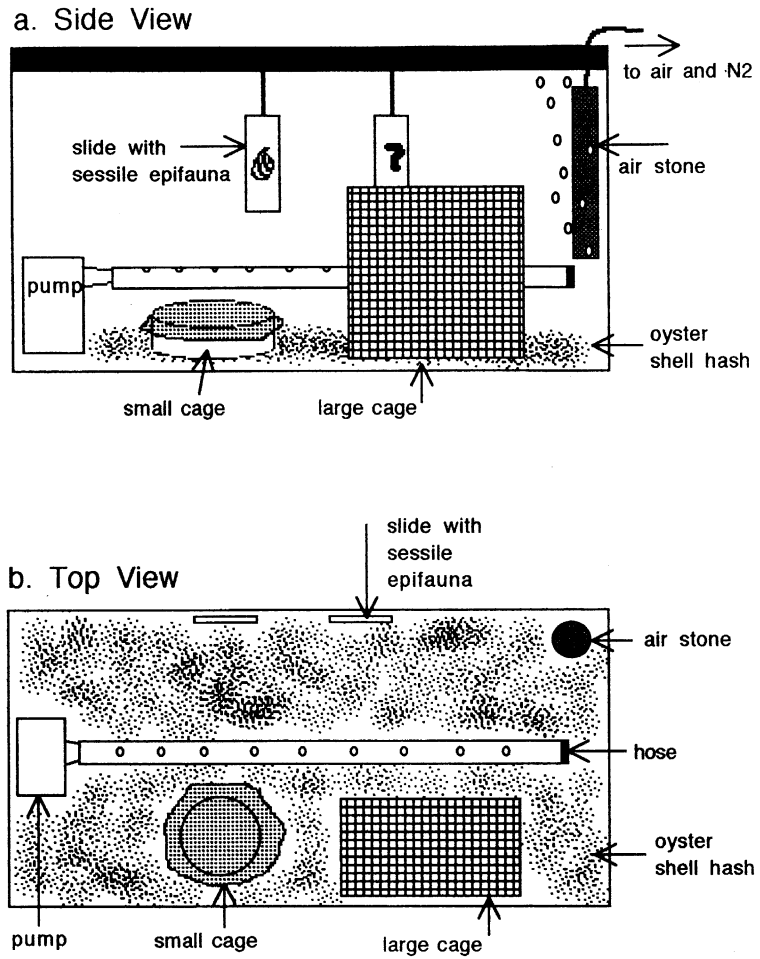

Fig. 1. (a) View of experimental aquaria from the side. Small and large cages housed mobile epifauna. (b) View of experimental aquaria from above.

tested are also found in deep areas of the York River where light is not present and where hypoxia occurs, and these were the conditions we attempted to mimic). To prevent hypercapnia, the build-up of CO_2, and subsequent pH changes (Burnett, 1997), 300–500 ml oyster shell hash buffered the pH in each tank (Fig. 1). Currents in the York River during hypoxia can reach 40 cm/s (Sisson et al., 1991), so submersible pumps in each aquarium maintained recirculating water flow of 400 l/h (1997) or 580 l/h (1998–1999). Each pump was connected to a 2.5 cm diameter plastic hose that ran down the center along the length of each aquarium (Fig. 1); water flowing through small holes spaced every 2.5 cm along the hose created relatively uniform water flow throughout each aquarium.

To ensure that water mixed throughout aquaria and flowed easily in and out of cages, we used dye tracers to examine water movement. Dye injected anywhere within an

aquarium dispersed throughout the aquarium within 5–10 s, suggesting that oxygen concentrations were relatively homogenous throughout aquaria. Similarly, dye injected in large cages completely dispersed out of cages in 5–10 s, suggesting that oxygen concentrations were likely to be similar inside and outside large cages. Dye in small cages showed a different pattern; dye in the upper half of the cages dispersed quickly around the aquaria (< 30 s), but dye in the bottom half remained stagnant. These results suggested that small cages had strong flow at the top but not the bottom. However, we believe animals in small cages experienced relatively similar oxygen conditions to those in the rest of the tanks for three reasons. First, animals in hypoxia usually clung to the top of cages where they would have experienced relatively high mixing with the rest of the tank. Second, animals in these cages were small so they could not have respired at a sufficiently high rate to change oxygen conditions drastically. Third, in 1999 we compared the mortality in small cages vs. a new set of cages (called "new cages"), with the same mesh size but different design. We made new cages using PVC pipe (2.5 cm inside diameter, 2.5 cm long), with 125 μm nytex mesh covering both ends. Dye in new cages dispersed into the rest of the tank within 10 s. In experiments with the polychaete *Ner. succinea*, we used half new cages and half old small cages. We observed nearly identical mortality in both cage-types (21–26 June 1999: four of five *Ner. succinea* died in small cages, four of five died in new cages; 15–20 August 1999: four of five *Ner. succinea* died in small cages, three of five died in new cages).

After placing animals in aquaria, we bubbled high-oxygen tanks with air to maintain oxygen concentrations above 4 mg O_2/l; low-oxygen tanks were bubbled with a combination of air and N_2 gas to achieve target oxygen concentrations (1 mg O_2/l in 1997; 0.5 mg O_2/l in 1998 and 1999) during the first 6 h of each experiment, a period over which similarly abrupt oxygen changes take place in the York River (Kuo and Neilson, 1987). Plastic wrap over the surface of each aquarium limited contact with the atmosphere. Oxygen and temperature in each aquarium were measured 6–10 times each day using a YSI model 38 oxygen monitor, calibrated daily. When oxygen differed from the target concentration, we changed the proportions of air and N_2 to correct the concentration. After 5 days, a typical duration for hypoxia in the York River (Kuo and Neilson, 1987), oxygen in low-oxygen aquaria was raised to > 4 mg O_2/l to let animals revive. Before raising oxygen, we measured the pH in each aquarium using a Beckman Φ220 pH meter or a Corning pH/ion analyzer 350.

To prevent the build-up of metabolic byproducts, each day we removed 20% of the water volume of each aquarium and replaced it by siphoning water from one of two randomly selected, oyster-shell buffered reserve tanks. Reserve tanks were filled with York River water and oxygen concentrations in each tank were decreased to < 1 mg O_2/l by bubbling with N_2 gas. After replacing water in low-oxygen tanks, we increased oxygen in the reserve tanks to > 3 mg O_2/l by bubbling with air and subsequently replaced water in high-oxygen tanks. Thus, all tanks used water that differed only in oxygen concentration.

Each species was fed an appropriate diet (Table 1). We fed suspension feeders a variety of foods (Table 1) including liquid filter feeder food (Liquefy Marine, from Interpet, five drops per aquarium daily), feeding blocks that dissolved over time (Reefcare Invertebrate Feeding Blocks, 0.5 block per aquarium, dissolved over the

course of each experiment), algae paste (diatom *Thalassiosira weissflogii*, approximately 0.5×10^8 cells per tank daily), brine shrimp (*Artemia* sp., < 6 h after hatching), or plankton (variable concentrations caught with a plankton net on the VIMS pier). Predators were offered epifaunal animals collected on the VIMS pier (Table 1). For most experiments, we placed predators and prey together in aquaria at the beginning of experiments so that both species experienced similar oxygen conditions throughout the experiment as they would in the natural environment. During two experiments with mud crabs *N. sayi* (7–12 June 1997; 23–28 June 1997), we placed predators and prey together in aquaria at the beginning of the experiment, then removed remaining prey items and added new ones after 2 and 3 days, respectively. Two species (the worm *Ner. succinea* and the snail *M. lunata*) were not fed because we could not provide an appropriate diet.

We observed all animals daily to determine if they were alive, using appropriate criteria for each species (Table 1), and removed dead animals. When possible, we observed animals without disturbing them. For a few species, it was necessary to observe animals using dissecting microscopes. In these cases, animals were placed in dishes with water from their own aquarium; to prevent oxygen from changing, we observed the animals quickly without jostling dishes. In all cases it took less than 10 min to observe animals and return them to aquaria. In experiments with *P. cornuta*, we observed a 0.5-cm^2 section, and counted tubes and the number of worms inside the tubes. During 1998 experiments with the bryozoan *Mem. tenuis*, we observed 30 zooids starting from the center of each colony and moving outward. In 1999 experiments with the bryozoans *Mem. tenuis* and *Con. tenuissium*, we placed colonies under a grid and observed 10 random zooids. We placed the hydroid *O. bicuspidata* beneath a grid and, using a dissecting microscope, observed the number of hydranths on 10 random upright stems. Some colonies lost all hydranths; we could not determine if these colonies were alive until after the experiment, when they were placed in high oxygen and observed for new growth.

Although it would have been ideal to test the tolerance of all species at both target concentrations (1 mg O_2/l in 1997; 0.5 mg O_2/l in 1998 and 1999), we did not have the resources to make this possible, and we could not always predict when species would be available for collection. As a result, five species were tested at both target concentrations, two species were tested only at 1 mg O_2/l, and nine species were tested only at 0.5 mg O_2/l. However, among the species tested at both 1 and 0.5 mg O_2/l, we found a consistent order of species when rated from least to most tolerant at each oxygen concentration, suggesting that, for at least some species, species with attributes that made them most tolerant of low oxygen 1 mg O_2/l were also highly tolerant of low oxygen at 0.5 mg O_2/l.

2.3. Feeding and predation

To examine the sub-lethal effect of hypoxia on feeding and predation, we observed species that ranged from highly tolerant (*O. bicuspidata*) to relatively intolerant (mud crabs, blue crab juveniles) of hypoxia and which included many of the most common small predators on epifaunal species (Sagasti et al., 2000). We fed the hydroid *O.*

bicuspidata a combination of brine shrimp and zooplankton (Table 1) dyed orange with non-toxic Eosin Y dye. Fifteen minutes after feeding each colony, we observed it using a dissecting microscope, placing colonies under a grid and observing 10 randomly selected upright stems. For each stem, we counted the number of hydranths and whether they had eaten (orange prey were visible through hydranths). For experiments with predators (mud crabs *N. sayi*, blue crab juveniles *C. sapidus*, flatworms *Sty. ellipticus*, and nudibranchs *D. obscura*) it was important to distinguish between prey that were eaten and those that died from low oxygen. For mud crabs feeding on the polychaete *Ner. succinea* (Table 1), the worm was considered eaten if it was missing from the cage. We fed barnacles *Bal. improvisus* to blue crabs, mud crabs and flatworms (Table 1). When barnacles died from hypoxia, they turned black within their shells but remained intact. When they were eaten by crabs, the shells were broken and empty. When barnacles were eaten by flatworms, the shells were intact but empty. For the nudibranch *D. obscura* preying on the bryozoan *Mem. tenuis* (24–29 August 1997), each aquarium had one cage with only bryozoans (predator excluded) and a second cage with bryozoans plus nudibranchs (predator present). On the third day of this experiment, we counted all bryozoan zooids that were healthy, that had died intact, or that were empty. All cages without predators had fewer than 2% empty zooids, while those with predators had up to 66% empty zooids; thus, we assume that most empty zooids were eaten.

2.4. Predation during *vs.* following hypoxia

It is possible that even if predators decrease feeding rates during low oxygen, they may compensate by increasing feeding rates when oxygen increases such that the prey species derives no net benefit from a low-oxygen predation refuge. To determine whether prey species could benefit from decreased feeding during hypoxia, we examined an extreme case with a relatively intolerant predator (mud crab *N. sayi*) and a relatively tolerant prey species (barnacle *B. improvisus*), because in this scenario the use of hypoxia as a predation refuge would be most likely. The experiment was similar to those described above, except that plastic containers ($10 \times 10 \times 6.3$ cm) were used instead of aquaria, and low oxygen concentrations (target 1 mg O_2/l) were only maintained for 2 days, after which oxygen was increased to > 5 mg O_2/l in all containers for an additional 3 days. In each container we placed one crab (8–15 mm carapace width) and 20 barnacles (2–4 mm basal diameter). Barnacles were fed with liquid filter feeder food (Liquefy Marine, from Interpet, one drop per container daily), and each day we determined whether barnacles were living, dead, or eaten.

2.5. Statistical analysis

For each species with greater than 1% mortality in low oxygen we calculated the median lethal time (LT_{50}) in hypoxia using maximum likelihood normit analysis (Newman, 1995). For each species, the cumulative proportion of animals that died in low-oxygen treatments each day was used in the analysis. When species had more than one animal per aquarium we calculated the proportion dead and used this proportion to calculate the overall proportion dead in hypoxic aquaria. We used Pearson Chi-squared

goodness-of-fit tests to determine if models fit the data for each species, and in all cases the data adequately fit the models (Newman, 1995). However, for species with little mortality during experiments, models could not predict 95% confidence intervals. For this reason, we could not provide confidence intervals for species with LT_{50} greater than 5 days. For species with less than 1% mortality in low oxygen, we did not calculate LT_{50}s but instead determined that they were highly tolerant.

To determine if oxygen affects feeding and predation, we used a variety of analyses to compare feeding and predation in high- vs. low-oxygen treatments. For the hydroid *O. bicuspidata*, repeated measures ANOVAs compared the number of hydranths per stem, and the percentage of hydranths that fed each day, in high vs. low oxygen conditions. Because hydroids feed with their hydranths, the number of hydranths per stem is a measure of colony feeding potential. The percentage of hydranths that fed is a more direct measure of whether individual polyps fed. For each analysis, assumptions of parametric statistics were checked using Cochran's test for homogeneity of variance (Underwood, 1997) and the Shapiro–Wilkes test for normality (Zar, 1996). To meet the assumptions for the number of hydranths per stem, we transformed the data using a log $(x + 1)$ transformation. The percentage of stems that fed were normally distributed and had homogenous variances, so these data were not transformed.

To determine if oxygen changed predation by common small predators, we used Mann–Whitney *U*-tests to compare the number of prey eaten by living predators in high vs. low oxygen. Because we only included data from living predators, sample sizes differed between treatments. For experiments with mud crabs *N. sayi* preying on the polychaete *Ner. succinea* (7–12 June 1997) or the barnacle *B. improvisus* (23–28 June 1997), we compared the number of prey eaten by crabs at the end of each feeding period (days 0–1 and 2–4 for *Ner. succinea*, days 0–3 and 4–5 for barnacles). In experiments with the flatworm *Sty. ellipticus* preying on the barnacle *B. improvisus* (23–28 June 1997), there was high mortality of flatworms, and there were two flatworms present in each cage. To have sufficient sample sizes and to minimize effects of flatworms that died on remaining flatworms, we compared only the cumulative number of barnacles eaten in high vs. low oxygen on days 0–2 by flatworms in cages where both flatworms survived. However, the trends we saw during the first 2 days continued for the rest of the experiment. For the nudibranch *D. obscura* preying on the bryozoan *Mem. tenuis*, we calculated predator effect by subtracting the percent of zooids that were empty in predator excluded cages from the percent empty with the predator present. Finally, we compared predator effect in high vs. low oxygen, considering only aquaria where the nudibranch survived until the third day of the experiment. In experiments with blue crab juveniles (*Cal. sapidus*) preying on barnacles *B. improvisus* (19–25 July 1998), we had high mortality of blue crabs, so we only considered feeding during the first 3 days, when many crabs were still alive. We compared the cumulative number of barnacles eaten in high vs. low oxygen on days 1–3 by crabs that survived until day 3.

To examine predation by mud crabs *N. sayi* on barnacles *B. improvisus* during vs. following hypoxia, repeated measures ANOVA related the number of barnacles eaten per day to oxygen treatment (low vs. high) and time (during vs. following hypoxia). Assumptions of parametric statistics were checked as above, and data were transformed by log $(x + 1)$ to meet assumptions.

3. Results

3.1. Physical conditions

During each experiment, oxygen in low-oxygen aquaria fell to target concentrations (1 mg O_2/l in 1997; 0.5 mg O_2/l in 1998–1999) during the first 6–10 h, while high-oxygen tanks remained above 4.5 mg O_2/l (Fig. 2). Oxygen fluctuated, but average concentrations in low-oxygen tanks remained within 0.2 mg O_2/l of target oxygen concentrations during 75% of measurements (Fig. 2). In each experiment, oxygen remained near target levels until the fifth day (Fig. 2). Temperatures ranged from 20.1°C to 26.7°C, similar to the temperature range encountered by York River epifaunal animals in summer (Sagasti et al., 2000). At the end of experiments, pH ranged from 7.5 to 8.2, suggesting that hypercapnia did not occur. There were no significant differences in pH between low-oxygen treatments and high-oxygen treatments at the end of any experiments (*t*-tests, $p > 0.05$).

3.2. Mortality

Hypoxia tolerance varied greatly among species, with percent mortality in low oxygen ranging from 0% to 100% (Table 2). Some species (2 of 7 at 1 mg O_2/l and 6 of 14 at 0.5 mg O_2/l) had LT_{50}s of less than 5 days, a typical duration for hypoxia in the York River, while others (3 of 7 at 1 mg O_2/l and 5 of 14 at 0.5 mg O_2/l) were so tolerant of low oxygen conditions (0.5–1 mg O_2/l) that they had no mortality during our experiments (Table 2). At 1 mg O_2/l (1997), the flatworm *Sty. ellipticus*, the nudibranch *D. obscura* and the mud crab *N. sayi* were among the least tolerant species, while the snail *M. lunata*, the anemone *Dia. leucolena* and the serpulid polychaete *H. dianthus* were highly tolerant (Table 2). At 0.5 mg O_2/l (1998–1999), *N. sayi*, the polychaetes *P. cornuta* and *Ner. succinea* and the blue crab *Cal. sapidus* had low survival, while all *D. leucolena*, *H. dianthus*, the hydroid *O. bicuspidata* and the polychaete *S. vulgaris* survived (Table 2). Percent mortality in high oxygen varied between 0% and 14%, and in 19 out of 25 species trials there was no mortality in high oxygen (Table 2).

Survival time decreased as oxygen fell for species tested at both target low-oxygen concentrations. For example, LT_{50} for the nudibranch *D. obscura* dropped from 4.65 days at 1 mg O_2/l to 2.98 days at 0.5 mg O_2/l; this difference was significant because 95% confidence intervals did not overlap. LT_{50} for the mud crab *N. sayi* dropped from greater than 5 days at 1 mg O_2/l to 1.31 days at 0.5 mg O_2/l (Table 2). Although we did not observe sufficient mortality to predict confidence intervals for *N. sayi* at 1 mg O_2/l, the trend is consistent with decreased survival as oxygen falls. One species, the snail *M. lunata* went from highly tolerant (no mortality) at 1 mg O_2/l to an LT_{50} of greater than 5 days at 0.5 mg O_2/l (Table 2). Although the confidence interval for *M. lunata* at 0.5 mg O_2/l is large, the data are again consistent with a trend of decreased survival in lower oxygen. The anemone *Dia. leucolena* and the serpulid polychaete *H. dianthus* were highly tolerant of both target concentrations; with no mortality at either low oxygen level.

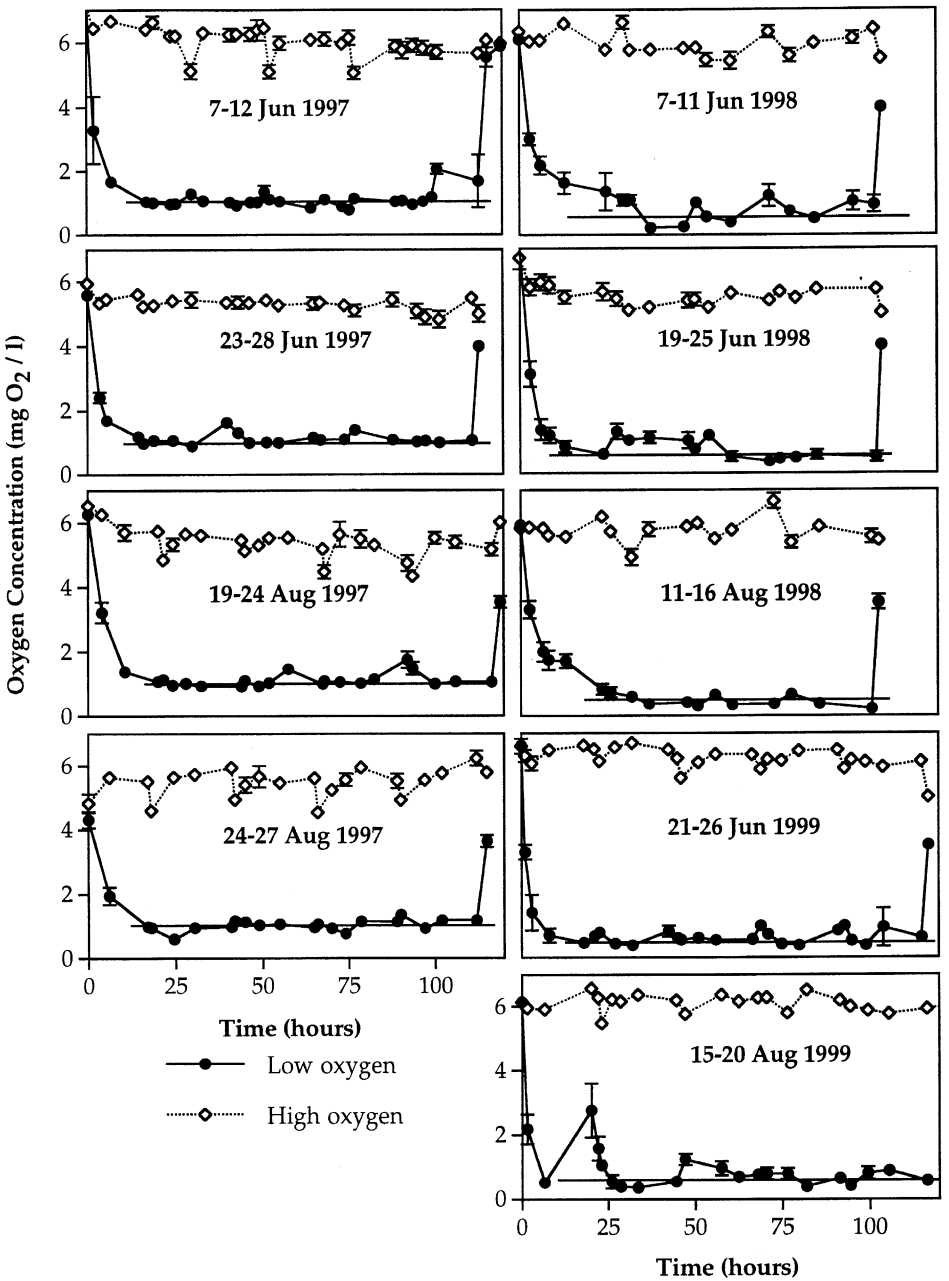

Fig. 2. Oxygen concentrations (mean ± 1 standard error) in aquaria during experiments, each of which lasted from 115 to 120 h (approximately 5 days). Reference lines mark target oxygen concentrations in low-oxygen treatments (0.5 or 1.0 mg O_2 /l). Some error bars are difficult to see because standard errors were small.

Table 2
Median Lethal Time (LT_{50}) of epifaunal species in low-oxygen treatments in 1997 (target oxygen concentration 1 mg O_2 /l) and 1998–1999 (target oxygen concentration 0.5 mg O_2 /l), and cumulative percent mortality of epifaunal species in low- and high-oxygen treatments

Species	$LT_{50\ (days)}$ (95% CI)	Percent mortality	
		Low oxygen	High oxygen
1997 (target oxygen concentration 1 mg O_2 / l)			
Sty. ellipticus	3.12 (2.13–5.44)	59	14
D. obscura	4.65 (3.41–58.06)	50	0
N. sayi, 7–12 June, 1997	> 5	40	0
N. sayi, 23–28 June, 1997	> 5	37	0
Cra. kaoruae	> 5	14	0
M. lunata, 23–28 June, 1997	tolerant to 5 days	0	0
M. lunata, 24–29 August, 1997	tolerant to 5 days	0	0
Dia. leucolena	tolerant to 5 days	0	0
H. dianthus	tolerant to 5 days	0	0
1998–1999 (target oxygen concentration 0.5 mg O_2 / l)			
N. sayi	1.31 (0.40–1.92)	90	0
C. sapidus	2.56 (1.91–3.18)	90	0
Ner. succinea, 21–26 June, 1999	2.57 (1.89–3.21)	80	0
Ner. succinea, 15–20 August, 1999	3.50 (2.75–4.67)	70	0
P. cornuta	2.89 (1.39–4.59)	90	4
D. obscura	2.98 (2.68–3.27)	100	3
Mol. manhattensis	4.49 (3.98–5.35)	70	0
M. lunata	> 5	45	0
B. improvisus	> 5	20	0
Mem. tenuis, 7–11 June, 1998	> 5	28	8
Mem. tenuis, 21–26 June, 1999	tolerant to 5 days	0.5	0.1
Con. tenuissium	tolerant to 5 days	0	0.5
Dia. leucolena	tolerant to 5 days	0	0
H. dianthus	tolerant to 5 days	0	0
O. bicuspidata	tolerant to 5 days	0	0
S. vulgaris	tolerant to 5 days	0	0

Some species were tested twice at a single target oxygen concentration; for these species we also include the date of each experiment. For species with LT_{50} greater than 5 days (the duration of the experiments), confidence intervals are large or the model was not able to compute them, so they are not listed. Species with no mortality during experiments are simply labeled tolerant to 5 days.

The order of species from least to most tolerant was similar but not identical at both target low oxygen concentrations. At both concentrations, the anemone *Dia. leucolena* and the polychaete *H. dianthus* were among the most tolerant, while the nudibranch *D. obscura* and the mud crab *N. sayi* were among the least tolerant (Table 2). However, *N. sayi* was more tolerant than *D. obscura* at 1 mg O_2/l, but less tolerant at 0.5 mg O_2/l (Table 2).

We tested some species twice at a single target low oxygen concentration to determine if LT_{50}s remained consistent, and found that although there was some variation between experiments for single species, but this did not change overall

patterns. For 3 out of 4 species (the mud crab *N. sayi*, the snail *M. lunata*, and the bryozoan *Mem. tenuis*), tolerance relative to other species did not change (Table 2). LT_{50}s during two separate experiments for the polychaete *Ner. succinea* at 0.5 mg O_2/l differed by 0.93 days, enough to change the order of its tolerance relative to the nudibranch *D. obscura* and the polychaete *P. cornuta*, but 95% confidence intervals of the two *Ner. succinea* trials overlapped (Table 2). Mortality for *Mem. tenuis* at 0.5 mg O_2/l did vary substantially between experiments, from 28% in 1998 to 0.5% in 1999, but these variations did not change tolerance relative to other species. Since mortality for *Mem. tenuis* in high oxygen was also much higher in 1998 (8%) than in 1999 (0.1%) it is possible that conditions were not ideal for this species during the 1998 trial.

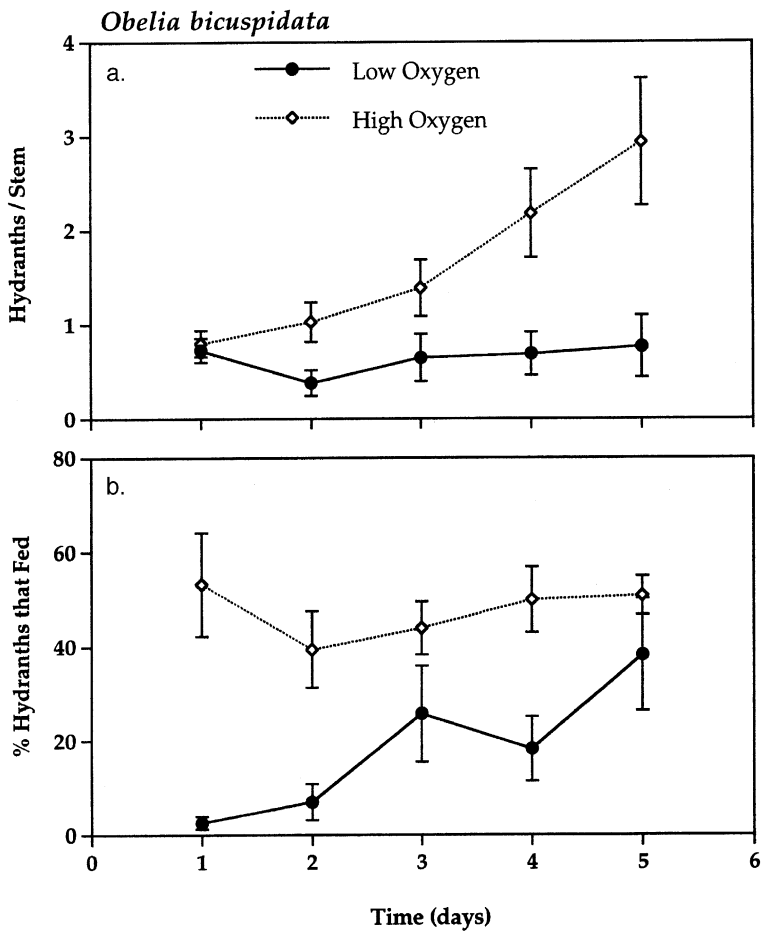

Fig. 3. (a) Number of *O. bicuspidata* hydranths/stem (mean ± 1 standard error) and (b) percent of hydranths (mean ± 1 standard error) that fed in low- and high-oxygen treatments in 1999.

3.3. Sub-lethal behavioral responses

Many species responded to low oxygen with distinctive behaviors that we did not observe in high oxygen. These behaviors remained consistent when a single species was tested in multiple years and in different oxygen concentrations. For example, in all experiments mobile species in low oxygen (crabs *N. sayi* and *Cal. sapidus*, flatworm *Sty. ellipticus*, snail *M. lunata*, nudibranchs *D. obscura* and *Cra. kaoruae*, and polychaete *Ner. succinea*) climbed to the tops of their cages, but in high oxygen we were just as likely to find them on the bottom or sides of cages. In addition, although *Cra. kaoruae* laid eggs throughout the experiment in high oxygen, it stopped laying eggs after 1 day in low oxygen. *Ner. succinea* remained on the cage side at all times in high oxygen, but in low oxygen they floated freely after 3 days. The tube worms *H. dianthus*, *P. cornuta* and *S. vulgaris* each partially left their tubes and extended their bodies into the water column. In low oxygen, the barnacle *B. improvisus* extended feeding appendages into the water column, but did not move them back and forth as if feeding, as they did in high oxygen. *Mol. manhattensis* elongated its body and siphons, so that siphons were higher above the substrate in low oxygen conditions. The anemone *Dia. leucolena* elongated its body and extended tentacles higher in the water column; subsequently it released its pedal disc from the substrate and floated, reattaching after oxygen concentrations increased. The bryozoans *Mem. tenuis* and *Con. tenuissimum* responded to hypoxia by forming brown bodies, a resting state that could help them decrease metabolism and wait until oxygen rose before resuming normal activities (Brusca and Brusca, 1990). Unfortunately, we did not record proportions of bryozoan

Table 3
Repeated measures ANOVA for the effect of oxygen treatment (high vs. low) and time on (a) the number of hydranths per stem and (b) the percentage of hydranths that ate, on each sampling date, for colonies of the hydroid *O. bicuspidata*

Source	DF	SS	MS	*F*	P
(a)					
Within subjects					
Time	4	0.918	0.229	5.43	0.0007
Time × oxygen	4	1.147	0.287	6.78	0.0001
Error	72	3.044	0.042		
Between subjects					
Oxygen	1	4.104	4.101	8.64	0.0088
Error	18	8.549	0.475		
(b)					
Within subjects					
Time	4	5201.742	1300.436	2.35	0.0622
Time × oxygen	4	4361.967	1090.491	1.97	0.1081
Error	72	39835.421	553.269		
Between subjects					
Oxygen	1	21138.252	21138.252	28.42	0.0001
Error	18	13387.048	743.725		

zooids that formed brown bodies, but this response occurred to some extent in all colonies in low oxygen, and was rare or absent from bryozoans in high oxygen. Although we observed clear rings of new zooids around all high oxygen *Mem. tenuis* colonies by the fourth day, we did not observe any growth of new zooids for *Mem. tenuis* in low oxygen.

3.4. Feeding and predation

Colonies of the hydroid *O. bicuspidata* started out with similar numbers of hydranths per stem in both oxygen treatments (Fig. 3). During the experiment the number of hydranths increased in high but not in low oxygen (Fig. 3). Repeated measures ANOVA shows a significant time × oxygen interaction (Table 3), because the difference between high and low oxygen increased with time (Fig. 3). At the end of the experiment, hydroids had grown more in high oxygen and had more hydranths with which to feed. The two treatments also differed in the percentage of hydranths that fed, with repeated measures ANOVA showing significantly higher percentages of hydranths feeding in high oxygen vs. low oxygen (Table 3).

The effect of oxygen on predation depended on the predator species and the timing of predation (Figs. 4–7). With two separate prey items (the polychaete *Ner. succinea* and the barnacle *B. improvisus*), the mud crab *N. sayi* showed decreased predation towards the end of experiments, but not at the beginning (Fig. 4). Low oxygen significantly decreased predation by flatworms *Sty. ellipticus* on the barnacle *B. improvisus* (23–28 June 1997) (Fig. 5). The nudibranch *D. obscura* showed significantly decreased predation on the bryozoan *Mem. tenuis* in low oxygen (Fig. 6). In contrast, blue crab

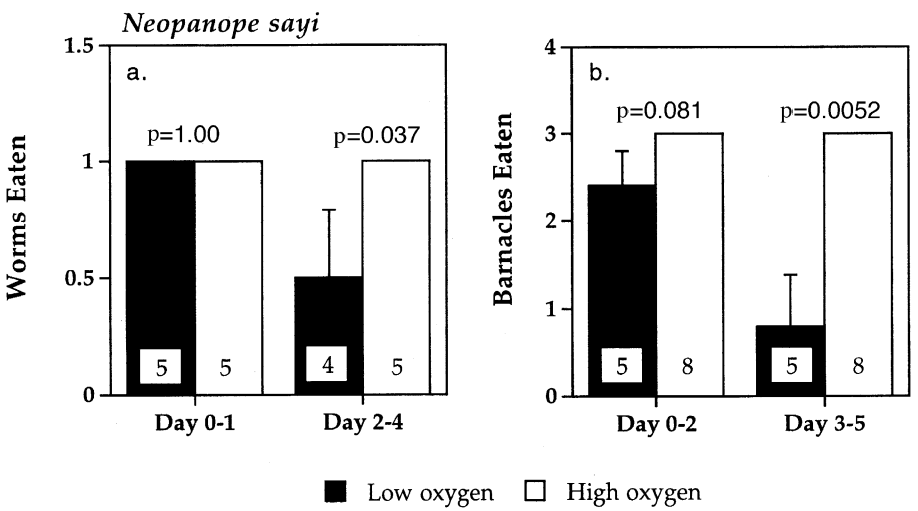

Fig. 4. (a) Cumulative number of worms *Ner. succinea* eaten on days 0–1 or 2–4 by the mud crab *N. sayi* in low- and high-oxygen treatments (mean ± 1 standard error) in 1997. (b) Cumulative barnacles *B. improvisus* eaten on days 0–2 or 3–5 by *N. sayi* in low- and high-oxygen treatments (mean ± 1 standard error) in 1997. Number of replicate crabs (n) are written in bars, and p-values are results of Mann–Whitney U-tests.

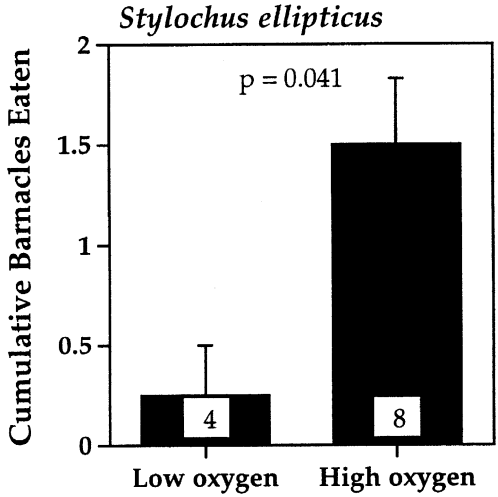

Fig. 5. Cumulative barnacles *B. improvisus* eaten by the flatworm *Sty. ellipticus* in low- and high-oxygen treatments (mean ± 1 standard error) in 1997. Number of replicate cages with two living flatworms (*n*) are written in bars, and *p*-value is the result of a Mann–Whitney *U*-test.

juveniles (*Cal. sapidus*) did not significantly change feeding rates on barnacles *B. improvisus* (19–25 July 1998) (Fig. 7). However, because so many blue crabs died during the experiment, more barnacles survived in hypoxia (52 of 60 barnacles in all

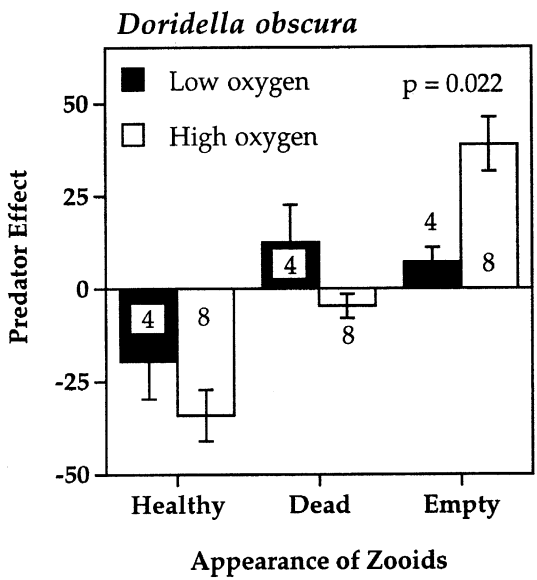

Fig. 6. Effect (percent in predator absent minus percent predator present) (mean ± 1 standard error) of the nudibranch *D. obscura* on zooids of the bryozoan *Mem. tenuis* in low- and high-oxygen treatments in 1997. Number of replicate nudibranchs (*n*) are written in bars, and *p*-value is the result of a Mann–Whitney *U*-test.

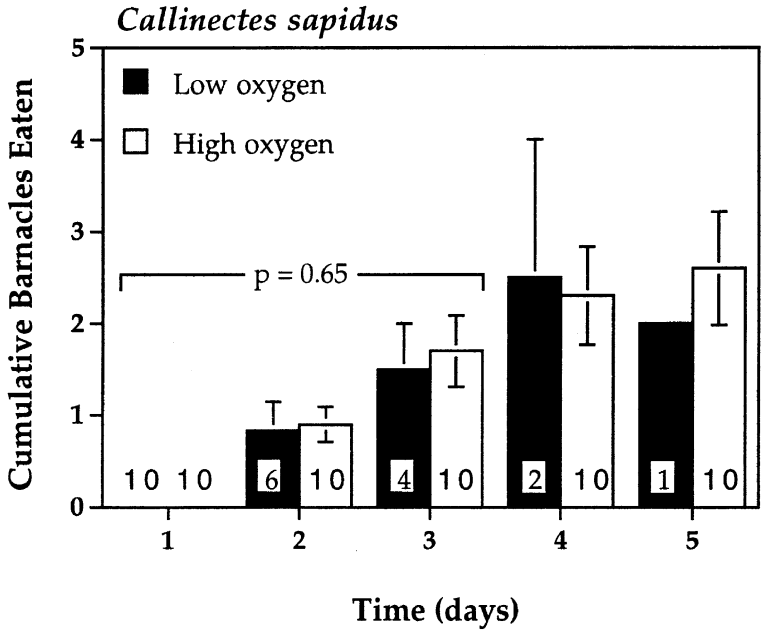

Fig. 7. Cumulative barnacles *B. improvisus* eaten daily by juvenile blue crabs (*C. sapidus*) in low- and high-oxygen treatments (mean ± 1 standard error) in 1998. Number of replicate crabs (*n*) are written in bars, and *p*-value is the result of a Mann–Whitney *U*-test comparing the cumulative odds of barnacles being eaten in days 0–3 in high vs. low oxygen.

replicates combined) than in normoxia (34 of 60 barnacles in all replicates combined) (Fig. 8).

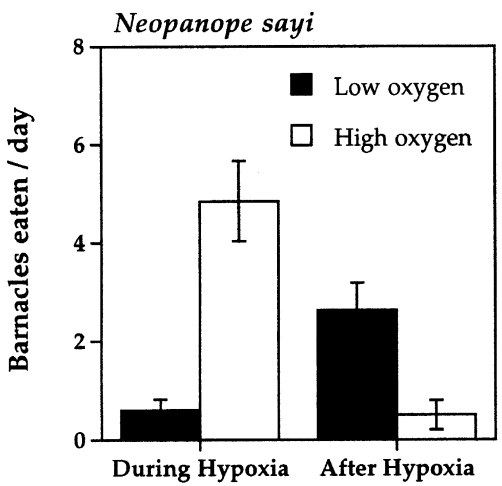

Fig. 8. Cumulative barnacles *B. improvisus* eaten daily by the mud crab *N. sayi* in low- and high-oxygen treatments (mean ± 1 standard error) during and following hypoxia. $n = 10$ crabs for all treatments.

Table 4
Results of repeated measures ANOVA for the effect of oxygen treatment (high vs. low) and time (during hypoxia vs. following hypoxia) on the number of barnacles eaten by mud crabs (*N. sayi*) per day

Source	DF	SS	MS	*F*	P
Within subjects					
Time	1	0.173	0.173	3.00	0.1003
Time × oxygen	1	2.149	2.149	37.30	0.0001
Error	18	1.037	0.058		
Between subjects					
Oxygen	1	0.081	0.081	2.04	0.1705
Error	18	0.715	0.040		

3.5. Predation during vs. following hypoxia

We observed no mortality of mud crabs (*N. sayi*) during this experiment, and less than 5% of barnacles (*B. improvisus*) died from exposure to hypoxia. Mud crabs in high oxygen consumed an average of 10 barnacles during the first 2 days and 12 barnacles by the end of the experiment (Fig. 8). In low oxygen, crabs ate only an average of one barnacle during the first 2 days; after oxygen levels were raised, though, they increased feeding rates so that they had consumed approximately nine barnacles by the end of the experiment (Fig. 8). ANOVA showed a significant time × oxygen interaction, presumably because during the first 2 days crabs ate more barnacles per day in high oxygen than in low-oxygen treatments, but during the last 3 days of the experiment, when oxygen was high in both treatments, crabs compensated for their energetic deficit by eating more barnacles per day in low-oxygen treatments (Table 4).

4. Discussion

Epifaunal species in the York River estuary have relatively high tolerance for hypoxia, that explains in part how they persist in areas periodically exposed to low oxygen (Sagasti et al., 2000). Many species had LT_{50}s greater than 7 days (3 of 6 species at 1 mg O_2/l and 6 of 14 species at 0.5 mg O_2/l) (Table 2), the maximum duration for typical hypoxic episodes in the York River (Kuo and Neilson, 1987). These species probably experience little or no mortality from low oxygen during typical hypoxic episodes in the York River. Species with shorter LT_{50}s could experience considerable mortality during hypoxic episodes, and yet they are still abundant in areas where hypoxia occurs (Sagasti et al., 2000). These species may survive in hypoxic areas for three reasons. First, oxygen in the York River fluctuates not only with the neap/spring tidal cycle, but also with daily flood and ebb tides (Kuo and Neilson, 1987). Therefore, epifaunal animals in hypoxic areas of the York River experience

hourly oxygen variation, and the lowest oxygen concentrations (near anoxia) generally last for only a few hours. Animals could experience frequent brief (hours) periods of normoxic (> 2 mg O_2/l) or mildly hypoxic (1.5–2 mg O_2/l) oxygen concentrations in the middle of severe hypoxic episodes (Diaz et al., 1992; Pihl et al., 1992), allowing them to recover. Conditions in our laboratory aquaria did not simulate these fluctuations, which may explain why we observed high mortality of some species in the laboratory, even though these species are abundant in areas of the York River where hypoxia occurs (Sagasti et al., 2000). Second, many species can re-colonize quickly after oxygen rises, or even recruit during hypoxia, allowing populations to re-establish themselves rapidly following hypoxic episodes (Sagasti et al., in preparation). Third, epifaunal species in the Chesapeake Bay grow quickly (Abbe, 1987). Many species can settle and reach maturity in just weeks (Abbe, 1987). These species may have sufficient time to grow between hypoxic episodes, particularly if they can survive some hypoxic episodes and grow during multiple periods of high oxygen.

The tolerance of oxygen stress by a given species varies with many factors including temperature, salinity (Herreid, 1980), sex (Gerhardt and Baden, 1998), age (Eriksson and Baden, 1997), season (Legeay and Massabuau, 2000), exposure to contaminants (de Zwaan and Eertman, 1996) and reproductive status (Vopel et al., 1998), so if we repeated this study under different conditions LT_{50} for some species might change. Consequently, the results of this study serve as a guide for comparing the relative tolerance of different species in this community to low oxygen but do not necessarily apply to all circumstances or habitats. For example, we did not expose animals to increased sulfide that may accompany hypoxia in the environment and which could have changed survival (Theede et al., 1969). However, hypoxia tolerance mirrors H_2S tolerance for many species, and high sulfide concentrations in the water column are only common in chronically anoxic environments (Theede et al., 1969). Epifauna in the York River rarely experience anoxia and so are relatively unlikely to encounter high H_2S. Our experiments also covered only one hypoxic pulse, not multiple pulses over the course of a growing season, which many animals may encounter in the field. It is possible that species that can tolerate single hypoxic pulses cannot survive repeated pulses, an issue which is beyond the scope of this paper but which should be investigated in the future. We believe our results reasonably approximate the relative hypoxia tolerance of epifaunal species in the York River for two reasons. First, we found consistent LT_{50}s when we tested a single species in multiple experiments, even though temperature, salinity, and the presence of other species differed among experiments. Second, the order of species when rated from least to most tolerant remained similar at different oxygen concentrations.

It is also possible that LT_{50}s of some tests were influenced by the presence of other species in some trials. If the presence of other species has strong effects on survival in hypoxia, then it might not be possible to compare the results of different experiments. However, as stated above, for species that were tested in multiple experiments at a single oxygen concentration, we found relatively consistent LT_{50}s, even though the presence of other species varied between these experiments. For example, at 1 mg O_2/l, the crab *N. sayi* experienced 40% mortality when they were the only species in aquaria, and 37% mortality when flatworms and snails were also being tested. *M. lunata*

experienced no mortality in aquaria with flatworms and mud crabs, in aquaria with nudibranchs, and in aquaria with blue crabs and nudibranchs. At 0.5 mg O_2/l, the polychaete *Ner. succinea* experienced 80% mortality in aquaria that also contained barnacles and bryozoans, and 70% mortality in aquaria that also contained the polychaete *S. vulgaris*, mud crabs, and hydroids. At least for these species, the presence or absence of other species did not have substantial effects on tolerance. One specie where other species could have had an effect may be the bryozoan *Mem. tenuis*, which, as stated above, showed considerable variation in mortality during two experiments at 0.5 mg O_2/l, one in which tunicates and the polychaete *P. cornuta* were also present, and one in which barnacles and another species of bryozoan, *Con. tenuissimum*, were also present, but these variations did not change tolerance relative to other species.

Hypoxia tolerance shows broad patterns among taxonomic groups, with polychaetes, mollusks, platyhelminths and cnidarians being relatively tolerant while crustaceans and vertebrates are relatively intolerant (Mangum and van Winkle, 1973; Diaz and Rosenberg, 1995). Nevertheless, tolerance of hypoxia can vary as much among species in a single taxonomic group as among species in different groups (McMahon and Russell-Hunter, 1978). In this study, we found high variability in tolerance among related species. For example, at 0.5 mg O_2/l, polychaete LT_{50}s varied from 2.57 days (*P. cornuta*) to highly tolerant with no mortality (*S. vulgaris* and *H. dianthus*), and among crustaceans, LT_{50}s at 0.5 mg O_2/l varied from 1.31 days (*N. sayi*) to 6.41 days (*B. improvisus*) (Table 2). For some taxonomic groups such as hydroids and bryozoans, this study is among the first to measure hypoxia tolerance. We found high tolerance of low oxygen for both hydroids and bryozoans (Table 2).

We found trends of decreased tolerance among mobile species relative to sessile species. For example, at 1 mg O_2/l, mobile species accounted for the five least tolerant species out of seven species tested, and at 0.5 mg O_2/l mobile species accounted for five of the seven least tolerant species out of 14 species tested. These trends are consistent with the findings of others in intertidal epifaunal communities exposed to wave stress, where mobile species were also the least tolerant (Menge, 1978; Menge and Sutherland, 1987), suggesting that mobile species may have decreased tolerance for physiological stresses as they do for physical stresses. These results also support consumer stress models (Menge and Sutherland, 1987; Menge and Olson, 1990), which suggest that mobile predators have lower tolerance of stress than sessile prey, leading to a decrease in the importance of predation in stressed communities.

Although many species showed little or no mortality in low oxygen, hypoxia changed behavior in all species, suggesting that hypoxia's greatest effects on epifauna in the York River may be through sub-lethal behavioral changes. As others have found for infauna, species responded to low oxygen with behaviors that allowed them to reach higher oxygen concentrations, escape hypoxic areas, or decrease metabolism (Hagerman, 1998). For example, all mobile animals in low oxygen clung to the tops of their cages. Presumably in nature these animals would climb shells, rocks or other structures to reach higher into the benthic-boundary layer where oxygen concentrations increase logarithmically with height above the bottom (Jørgensen, 1980; Diaz and Rosenberg, 1995). Many sessile species also reached higher into the water column. Presumably, reaching higher oxygen concentrations in the water column could allow animals to transport oxygen

back to tissues attached to the bottom. Animals that stretch out into the benthic boundary layer also benefit from increased exposure to flow that reduces their diffusive boundary layer thickness, thus increasing the flux of oxygen to their bodies. The anemone (*Dia. leucolena*) elongated its body and extended it higher into the water column, then eventually released its pedal disk and floated. Sassaman and Mangum (1972) hypothesized that anemones elongate in hypoxia to increase their surface area to volume ratio, thus increasing the flux of oxygen to tissues. Sassaman and Mangum (1972) did not observe anemones releasing their pedal discs; possibly this behavior does not occur in the species they investigated. In our experiments, *Dia. leucolena* reattached to hard substrates after oxygen increased, thus, floating away from hypoxic areas may represent an escape behavior. The polychaete *Ner. succinea* also floated into the water column after prolonged hypoxia, and may escape hypoxic areas by floating away. Other species responded to low oxygen with behaviors that may decrease metabolism, such as entering resting states (bryozoans) and decreasing reproductive activities (nudibranch *Cra. kaoruae*).

Although these sub-lethal effects stopped after oxygen increased, in our experiments, they disrupted normal behaviors for several days. Most Chesapeake Bay epifaunal species live only one season (Abbe, 1987), so short disruptions in an animal's normal behaviors could disrupt a considerable portion of that animal's lifetime and make it less likely that they could reproduce. In the York River, hypoxic episodes occur several times during each growing season, and benthic animals are thus likely to experience sub-lethal hypoxic effects throughout their lifetimes.

A further sub-lethal effect of hypoxia in some epifaunal species may be decreased feeding and growth, which could lead to changes in competitive abilities. For example, growth of new zooids occurred on the edges of colonies of the bryozoan *Mem. tenuis* in high but not low oxygen. The hydroid *O. bicuspidata* was among the most tolerant species in this system, with no mortality during our experiments, yet *O. bicuspidata* showed substantially decreased growth in low oxygen (Fig. 3, Table 3). *O. bicuspidata* hydranths were also significantly less likely to feed in low oxygen, which presumably contributed to the decreased growth. Many sessile epifaunal species compete with other species for space and capture space by lateral growth of colonies (Jackson, 1979). Therefore, decreased growth during hypoxia may reduce the ability of some species to capture space and compete. However, epifaunal species in the Chesapeake Bay have high growth rates (Abbe, 1987) and may be able to compensate for slow growth during hypoxia by growing rapidly between hypoxic episodes. In other words, hypoxic stress may be too infrequent relative to growth rates of the common species to affect community structure appreciably (Huston, 1979).

It is likely that hypoxia decreases predation by small epifaunal predators on other epifaunal species for two reasons. First, low oxygen decreased predation rates of mud crabs *N. sayi*, flatworms *Sty. ellipticus* and nudibranchs *D. obscura*. These species are among the most abundant predators in this community (Sagasti et al., 2000) and have major effects on population abundance and community structure in Chesapeake Bay epifaunal communities (Branscomb, 1976; Marsh, 1976; Rheinhardt and Mann, 1990). Second, predators were among the species least tolerant of hypoxia, so it is possible that many of them die during hypoxic episodes. Thus, even though juvenile blue crabs (*Cal.*

sapidus) did not decrease feeding rates on barnacles, so many crabs died in hypoxia that fewer barnacles were eaten.

Decreased predation during hypoxia could lead to species using hypoxic episodes as predation refuges. This may be especially true because many prey species were more tolerant of hypoxia than their predators and would experience lower direct mortality from hypoxia. However, when we compared predation during vs. following hypoxia for a highly tolerant prey (barnacles *B. improvisus*) and an intolerant predator (mud crabs *N. sayi*), we found that, although crabs had decreased feeding during hypoxia, they increased feeding following hypoxia. Therefore, prey in areas with hypoxic episodes may only have temporary reprieves from predation during hypoxic episodes, after which predation catches up to that in high-oxygen areas. It is likely that the use of hypoxic areas as predation refuges depends on the relative tolerance of predators and prey, and on the duration of hypoxia. During short hypoxic episodes, predators may temporarily decrease feeding but then increase feeding after oxygen increases, leading to no net benefit for the prey. During longer hypoxic episodes, predators could have decreased feeding and high mortality, leading to reduced mortality for prey (but this refuge may be compromised by lower prey growth during hypoxia). Finally, prolonged hypoxia may lead to death of both predators and prey.

Unlike other systems where hypoxia tolerance has major effects on species distributions (Prasada Rao and Ganapati, 1968; McMahon and Russell-Hunter, 1978; Marshall and McQuaid, 1993; Nielsen and Hagerman, 1998), the distribution of epifaunal species in the York River did not correlate with hypoxia tolerance (Sagasti et al., 2000), suggesting that hypoxia is not a major factor determining distributions in this system. If hypoxia tolerance controlled distributions, we would expect to find increased abundance of the most tolerant species and decreased abundance of intolerant species in areas with the lowest oxygen. However, in a companion study (Sagasti et al., 2000) we found that basins in the York River where hypoxia is most severe have greatly increased abundances of the polychaete *P. cornuta*, a species that in this study was among the most susceptible to hypoxia. Areas with lowest oxygen also had decreased abundances of the polychaete *S. vulgaris* and the bryozoans *Mem. tenuis* and *Con. tenuissimum*, species that were highly tolerant of hypoxia in this study.

The York River epifaunal community appears to be highly resistant to low oxygen stress for several reasons. First, many species can survive hypoxia for over a week, and the York River experiences relatively short hypoxic episodes (up to 7 days). Second, species have behavioral mechanisms that allow them to increase oxygen flux to their bodies, such as reaching higher into the water column. Third, species with high mortality during low oxygen may maintain populations with high recruitment, which can occur even during hypoxic events (Sagasti et al., in preparation). Finally, although many species reduce feeding and predation during hypoxia, leading to reduced growth, high food availability in the Chesapeake Bay (Kemp et al., 1997) may allow species to grow quickly between hypoxic episodes. In summary, the species in this community cannot only withstand hypoxic stress but also recover quickly between hypoxic episodes. These characteristics may become even more important as increased eutrophication increases hypoxic stress in this system (Diaz and Rosenberg, 1995).

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