

Physiological Responses to Hypoxia

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Abstract

Hypoxia can have profound effects on individual organisms. This chapter focuses on the mechanisms different kinds of animals possess to avoid, tolerate, and adapt to low levels of oxygen in water; selected examples illustrate these mechanisms. While some organisms can detect and avoid hypoxic water, avoidance is not always possible, especially in the case of sessile organisms. When an organism cannot avoid hypoxia, its response may depend on the intensity and the duration of the bout of low oxygen. Examples of responses to hypoxia include a depression in feeding as well as a decrease in molting and growth rates. During acute exposures to hypoxia some organisms can maintain aerobic metabolism by making effective use of a respiratory pigment, or increasing ventilation rates, or increasing the flow of blood past the respiratory surfaces or combinations of all three. Responses to chronic hypoxia are different and include the production of greater quantities of respiratory pigment and changing the structure of the pigment to one with an adaptive higher oxygen affinity. Many organisms respond to hypoxia by switching from aerobic to anaerobic metabolism and some simply reduce their overall metabolism. Hypoxia is often accompanied by hypercapnia (an elevation in water CO_2), which produces an acidification of the body tissues, including the blood, and has physiological implications that can also be profound and separate from the effects of low oxygen. Finally, there is evidence that hypoxia can inhibit immune responses, causing greater mortality than would otherwise occur when organisms are challenged with a pathogen.

Introduction

An obvious and dramatic effect of low ambient oxygen on an organism is a lethal response. The general public is well aware of the results of hypoxia when large “fish kills” are reported. However, organisms can be affected by a lack of oxygen in other ways.

Coastal Hypoxia: Consequences for Living Resources and Ecosystems
Coastal and Estuarine Studies, Pages 101-114
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Hypoxia may limit the energy budget or scope for growth and activity of an organism, it may cause an organism to alter its behavior, and/or it may limit the tolerance of an organism to other environmental challenges. The manifestations of these effects may be seen as changes in the population structure within a species, changes in the range of distribution, or a decrease in the population density of an organism. In this manner individual organismal effects are transferred to the population and ecosystem levels of organization. From a population perspective, there is a big difference between the ability of an organism simply to survive and its ability to thrive. This difference can be evidenced in the structure of food webs and the size distribution of organisms within a population. In this chapter, we explore some of the individual organismal responses to hypoxia and the effects of hypoxia on basic physiological mechanisms and behavior. The examples we use are selected from the literature based on their relevance to the kinds of organisms living in the Gulf of Mexico and are summarized in Table 1. In addition, we discuss some new information that suggests that resistance to disease is compromised in hypoxic environments.

Environmental hypoxia can be moderate (e.g., half air saturation) or severe (e.g., less than 20 to 30% air saturation), designations not used consistently in the literature. Obviously, the more severe the hypoxia, the greater the physiological challenge to the organism. More severe hypoxia may, for example, require an organism to utilize anaerobic metabolism to sustain its energy production. Another water quality variable that nearly always accompanies hypoxia is elevated carbon dioxide or hypercapnia [Cochran and Burnett, 1996; Burnett, 1997]. The biological demand for oxygen responsible for lowering oxygen partial pressures produces carbon dioxide as the main product of metabolism and even slight hypercapnia lowers water pH dramatically. Even though marine systems are considered to be well buffered against changes in pH, significant fluctuations in carbon dioxide occur in coastal waters resulting in highly variable water pH [Christmas and Jordan, 1987; Cochran and Burnett, 1996; Burnett, 1997]. Thus, aquatic organisms facing a drop in dissolved oxygen also face an acidification that causes a concomitant acidosis in the blood and the tissues. Below, we examine some of the consequences of hypercapnic hypoxia.

The duration of exposure to environmental hypoxia may be relatively short and diurnal or tidal [Summers et al., 1997; Spicer et al., 1999; Das and Stickle, unpublished observations] or it may be long term exposure for weeks to years [Stickle et al., 1989; Das and Stickle, 1993]. Adaptations to hypoxia likewise vary depending on the duration of exposure. Different response patterns to diurnal hypoxia compared to chronic hypoxia of the estuarine crab *Callinectes sapidus* and the offshore crab *C. similis* highlight these metabolic, feeding, and growth rate adaptations [Das and Stickle, 1993, unpublished observations] and are discussed below.

Thus, in this chapter we explore examples of the responses of organisms to hypoxia that illustrate the state of our knowledge and the problems organisms face. In addition, we suggest some areas for future research.

Physiological and Behavioral Responses

Many organisms living in coastal environments are well adapted to endure and even thrive for short durations in hypoxic water. Organisms are able to exist for short durations in hypoxia because they possess respiratory mechanisms to take up oxygen from the

TABLE 1. Behavioral and physiological responses of different organisms to hypoxia.

Organism	Response to Hypoxia	Reference
Shrimp		
<i>Penaeus aztecus</i>	detect and avoid	Renaud, 1986
<i>Penaeus setiferus</i>	detect and avoid	Renaud, 1986
<i>Penaeus monodon</i>	decrease hemocyte phagocytosis	Direkbusarakom & Danayadol, 1998
<i>Penaeus stylirostris</i>	decrease total hemocyte count increased mortality induced by <i>Vibrio alginolyticus</i>	Le Moullac et al., 1998 Le Moullac et al., 1998
Crabs		
<i>Callinectes sapidus</i>	detect and avoid decrease feeding reduce growth rate	Das & Stickle, 1994 Das & Stickle, 1993 Das & Stickle, 1993
	Acute Hypoxia	
	increase ventilation rate	Batterton & Cameron, 1978
	increase heart rate	deFur & Pease, 1988
	slight increase in cardiac output	deFur & Pease, 1988
	Chronic Hypoxia	
	decrease oxygen consumption	Das & Stickle, 1993
	no change in ventilation	deFur & Pease, 1988
	no change in heart rate	deFur et al., 1990
	increase hemocyanin O ₂ affinity and concentration	
<i>Callinectes similis</i>	detect and avoid increase oxygen consumption decrease feeding	Das & Stickle, 1994 Das & Stickle, 1993 Das & Stickle, 1993
Gastropod Molluscs		
<i>Stramonita haemastoma</i>	reduce growth rate large reduction in metabolism decrease oxygen consumption	Das & Stickle, 1993 Liu et al., 1990 Das & Stickle, 1993
Bivalved Molluscs		
<i>Crassostrea virginica</i>	switch to anaerobic metabolism small reduction in metabolism decrease production of reactive oxygen species	Stickle et al., 1989 Stickle et al., 1989 Boyd & Burnett, 1999

environment when it is scarce or because they sustain energy production by switching to anaerobic biochemical pathways. Many organisms can do both. Yet another option is to lower the overall rate of metabolism. However, if an organism is to sustain its "normal" rate

of activity, it must maintain its energy production. This may be especially important if the levels of dissolved oxygen fluctuate on a tidal and a diurnal basis as they often do [Cochran and Burnett, 1996; Das and Stickle, unpublished observations]. Finally, some organisms appear to make physiological adjustments when confronted with hypoxia on a chronic basis. Examples of these responses are presented below.

Avoidance Behavior

Perhaps the first line of defense of a mobile organism against hypoxia is to avoid it. A number of coastal species appear to be able to detect and avoid hypoxia. Investigations in the field have documented migration of fishes, crustaceans and annelids in response to low oxygen [Loesch, 1960; May, 1973; Garlo et al., 1979; Pavela et al., 1983; Pihl et al., 1991; Nestlerode and Diaz, 1998]. Differential predator and prey activity in hypoxic water can enhance feeding by the predator species [Nestlerode and Diaz, 1998]. Careful laboratory studies have supported these observations. Two such cases are mentioned below.

Juvenile brown shrimp, *Penaeus aztecus*, and juvenile white shrimp, *Penaeus setiferus*, can detect and avoid hypoxia [Renaud, 1986]. Furthermore, a number of behavioral responses are associated with hypoxia. These responses include an initial increase in activity, retreat from the hypoxia by walking or swimming, rapid eye-stalk movements, and flexing of antennal scales. The non-homogeneous patterns of hypoxia and well oxygenated water along the Gulf coast are thought to cause brown and white shrimp to aggregate in areas that are less hypoxic. Renaud [1986] suggests that this crowding could lead to increased animal stress and greater predation.

Juvenile blue crabs (both *Callinectes sapidus* and the lesser blue crab *Callinectes similis*) are also able to detect hypoxia [Das and Stickle, 1994]. Both species of blue crabs prefer water greater than two-thirds air saturation, and both species are more active in water of higher oxygen pressures than in hypoxic water [Das and Stickle, 1994]. Interestingly, unlike *C. similis*, juvenile *C. sapidus* do not avoid hypoxic water. Upon exposure to hypoxia, blue crabs demonstrate behaviors similar to that of shrimp (see above). They exhibit "restless and erratic movements" when exposed to hypoxia and move their eye-stalks with a greater frequency [Das and Stickle, 1994]. They also rapidly move their antennae. In addition, *C. sapidus* and *C. similis* exhibit decreased feeding rates in hypoxic water. There was a dramatic decline in growth rate as a function of the severity of hypoxia in *C. sapidus*, but not in *C. similis*. The reduced growth of *C. sapidus* was seen even with mild hypoxia, i.e., 74% air saturation [Das and Stickle, 1994].

Obviously, hypoxia has profound effects on the behavior of shrimp and crabs, even though they have physiological and biochemical mechanisms that enable them to cope with it (see below). That these responses include a decrease in feeding and growth rates suggests why populations of organisms, although they may survive hypoxia, do not thrive.

A behavioral pattern that is stronger than hypoxia avoidance in crustaceans is the diel vertical migration of Nordic krill, *Meganyctiphanes norvegica*, into depths that are severely hypoxic (> 70 m depth) in Gullmarsfjord, Sweden during daylight [Spicer et al., 1999]. Krill trawled at dusk had hemolymph lactate concentrations that were similar to lactate concentrations of krill caged at 70 m depth for the day, indicating that migrating krill had also undergone anaerobic metabolism during the day.

Effects of Hypoxia on Physiological Functions

Little is known of how acute or chronic hypoxia affects activity and growth in organisms. Chronic hypoxia (28 days exposure) results in a reduction in growth rate, as measured by Scope for Growth in the blue crab, *Callinectes sapidus*, the lesser blue crab, *C. similis* and the southern oyster drill, *Stramonita haemastoma* [Das and Stickle, 1993]. Feeding rate, the primary determinant of Scope for Growth [Stickle, 1985], was reduced in blue crabs exposed to severe hypoxia and varied directly with the severity of hypoxia in the southern oyster drill. Oxygen consumption rates of blue crabs and southern oyster drills exposed to severe hypoxia were significantly lower than in animals exposed to normoxia. In contrast, the oxygen consumption rate of *C. similis* was higher in hypoxia than in lesser blue crabs exposed to normoxia, indicating overcompensation in the oxygen delivery system. Growth and molting rates in both species of crabs were significantly higher in the normoxic exposure than in crabs exposed to hypoxia.

Exposure of these species to 28 days of a 100-16-100% diurnal pattern of air saturation produced bioenergetic results that differed from the earlier chronic exposure to various levels of dissolved oxygen [Das and Stickle, unpublished observations]. There was 3% mortality in *C. sapidus*, 11% in *C. similis*, and 0% in *S. haemastoma* exposed to this fluctuating pattern of diurnal variation in oxygen tension [Das and Stickle, unpublished observations]. The feeding rate of *C. sapidus* exposed to a fluctuating diurnal pattern of hypoxia was significantly higher than that of blue crabs exposed to constant 100 or 16% saturation. However, the feeding rate of *C. similis* exposed to diurnal hypoxia was lower than that of lesser blue crabs exposed to 100% oxygen saturation or 16% oxygen saturation. Both species of crabs exposed to the diurnal pattern of oxygen variation increased in weight during the experimental period at a rate faster than those exposed to 16% oxygen saturation, but not significantly different than crabs exposed to normoxia. Growth rate of *C. sapidus* exposed to the diurnal variation of oxygen tension was significantly higher (95% increase in wet weight) than that of *C. similis* (73% increase in wet weight).

A Case Study of Mechanisms Organisms Use to Handle Hypoxia

Even though organisms may have the ability to detect and avoid hypoxia, they may not always escape it. In this case, organisms must rely on physiological mechanisms to extract as much oxygen as possible from the water and transport it to the tissues or switch to anaerobic metabolic pathways to supply energy, or both. A number of organisms are able to maintain oxygen uptake when oxygen supplies in the ambient environment are limiting. The term "oxygen regulation" is often applied to this phenomenon. The ability to maintain or regulate oxygen uptake is obviously important in allowing an organism to sustain its normal scope of activity.

One of the best known examples of organisms that tolerate and respond to hypoxia is found in the blue crab, *Callinectes sapidus*. Blue crabs are highly active animals that are known to tolerate a wide range of temperatures, salinities, and oxygen conditions. This tolerance allows them to exploit a wide range of habitats. Adult *C. sapidus* are able to tolerate moderate hypoxia (i.e., 32% air saturation) quite well for 25 days with only 20% mortality [deFur et al., 1990]. In contrast, juvenile *C. sapidus* exhibit 50% mortality at 68%

air saturation after 28 days exposure [Das and Stickle, 1993]. Juvenile *C. sapidus* are also poor regulators of oxygen uptake when exposed to severe hypoxia, but *C. similis* exhibit elevated oxygen consumption rates relative to normoxia at all hypoxia levels analyzed [Das and Stickle, 1993].

The responses to short-term hypoxia exposure in adult blue crabs are similar to those found in other crabs. Many crabs respond to short-term hypoxia by increasing the ventilatory flow of water past the gills [Truchot, 1975; Burnett, 1979; Burnett and Johansen, 1981; Lallier and Truchot, 1989], which favors the diffusion of oxygen into the blood. Blue crabs acutely exposed to hypoxia (one-third air saturation) hyperventilate [Batterton and Cameron, 1978]. During chronic exposure to hypoxia, hyperventilation persists for five days and then returns to the normoxic baseline [deFur and Pease, 1988]. Hyperventilation often produces a respiratory alkalosis in crabs [Burnett and Johansen, 1981], elevating the hemolymph pH and thus giving rise to an adaptive increase in the oxygen affinity of hemocyanins with normal Bohr shifts. This alkalosis associated with hypoxia has been observed in *C. sapidus* [Pease et al., 1986]. Heart rate increases by as much as 30%, remains elevated for five days and returns to the normoxic baseline despite the persistence of hypoxia [deFur and Pease, 1988]. While cardiac output increases during hypoxia in the lobster *Homarus americanus* [McMahon and Wilkens, 1975] and the spider crab *Libinia emarginata* [Burnett, 1979], it increases by only a small amount in blue crabs, despite the increase in heart rate [deFur and Pease, 1988].

Long-term exposure to moderate hypoxia has been well studied in the blue crab [Stickle et al., 1989; deFur et al., 1990; Das and Stickle, 1993]. After 25 days of exposure of adult blue crabs to moderate hypoxia ($P_{O_2} = 50\text{--}55$ torr or 33% air saturation), oxygen uptake is no different from that of crabs living in well-oxygenated water [deFur and Pease, 1988]. Furthermore, cardiac output was only slightly elevated. Most of the adjustments that account for sustaining oxygen uptake during chronic hypoxia occur with hemocyanin. Small increases in hemocyanin oxygen affinity (i.e., decreases in P_{50}) occur as a result of changes in hemolymph lactate, urate, and calcium ion concentrations.

The elevated water CO_2 that accompanies hypoxia [Cochran and Burnett, 1996] results in elevated hemolymph CO_2 in blue crabs [Cameron, 1978]. Carbon dioxide, independent of pH, increases oxygen affinity [Mangum and Burnett, 1986], contributing to the adaptive response. These changes comprise extrinsic factors that interact with the hemocyanin molecule to modify its oxygen affinity. Extrinsic factors generally include ions such as hydrogen, calcium, magnesium and sodium, or organic molecules such as lactate and urate. Taken together, the extrinsic factors described above result in an increase in oxygen affinity of approximately 3 torr.

Chronic hypoxia stimulates significant changes in the concentration and the structure of the hemocyanin molecules of *C. sapidus* [Mangum, 1997]. Hemocyanin concentration increases by about 40%, enhancing the capacity of the hemolymph to carry oxygen. Hemocyanin levels also increase in the shrimp *Crangon crangon* during prolonged exposure to hypoxia [Hagerman, 1986]. But perhaps more interesting is the intrinsic adaptation of hemocyanin oxygen affinity to low oxygen. deFur et al. [1990] postulated that the net synthesis or degradation of hemocyanin during hypoxia produces replacement molecules that differ from those in normoxic crabs. This possibility becomes even greater given the net synthesis of hemocyanin that occurs during long-term hypoxia [Senkbeil and Wriston, 1981].

Intrinsic changes in the structure of hemocyanin can alter the oxygen affinity in response to chronic changes in environmental variables in crayfish [Rutledge, 1981] and crabs [Mauro and Mangum, 1982; Mason et al., 1983; Mangum and Rainer, 1988]. There are six different kinds of subunits that make up the large hemocyanin molecule in *C. sapidus* and these can be distinguished electrophoretically [Mangum and Rainer, 1988]. Three of the six subunits are known to be variable in different populations of blue crabs [Mangum and Rainer, 1988]. Subunits 3, 5, and 6, the variable subunits, decrease in concentration in relation to the other subunits in response to chronic hypoxia. The net result of the changes in subunit composition of hemocyanin is an overall increase in oxygen affinity (decrease in P_{50}) of 5 torr. It appears that the electrophoretic patterns observed in both the field and the laboratory bring about a higher hemocyanin oxygen affinity by favoring the more primitive subunits of hemocyanin [Mangum, 1997].

The Role of Anaerobic Metabolism

For many organisms avoidance of hypoxia is not an option. The eastern oyster, *Crassostrea virginica*, is obviously not able to avoid hypoxic water. The mechanisms it uses to cope with hypoxia are fundamentally different from those used by organisms such as the blue crab. *C. virginica* is a weak oxygen regulator [Galtsoff and Whipple, 1931; Shumway and Koehn, 1982; Willson and Burnett, 2000]. Like many bivalves it has well developed biochemical pathways to sustain energy production anaerobically [Gäde, 1983]. Using microcalorimetry, Stickle et al. [1989] showed that oysters maintained 75% of their normoxic energy consumption in water that was extremely hypoxic (< 5% air saturation). At this same oxygen level, oxygen uptake is approximately 10% of that in well-aerated water [Willson and Burnett, 2000]. Therefore, in severely hypoxic water most of the energy production is via anaerobic pathways with the remainder contributed by aerobic means.

Another metabolic pattern is found in the southern oyster drill, *S. haemastoma*; metabolism declines in environments with acutely declining oxygen levels. *S. haemastoma* is a poor regulator of oxygen uptake [Kapper and Stickle, 1987]; the index of oxygen regulation [Mangum and Van Winkle, 1973], B2 is -0.073×10^{-3} at 30°C and 30‰ S. Furthermore, there is no change in the response to ambient oxygen when snails are held for 28 days at $P_{O_2} = 53$ torr (34% air saturation) [Kapper and Stickle, 1987]. Although *S. haemastoma* is capable of switching from aerobic to anaerobic metabolism, it does not rely on this strategy during anoxia (i.e., oxygen < 5% air saturation). Rather, it reduces its overall metabolism. This has been demonstrated in calorimetric studies in which Stickle et al. [1989] showed a steady state rate of heat dissipation of 0.73 joules g dry weight⁻¹ h⁻¹ under anoxia, or 9% of the rate during normoxia (8.76 joules g dry weight⁻¹ h⁻¹). This contrasts with a normoxic energy consumption of 75% of the normoxic rate in the oyster, *C. virginica* under similar conditions (above).

Although highly active species such as blue crabs are capable of anaerobic metabolism, and accumulate L-lactate, they do not tolerate hypoxia to the extent of organisms like *C. virginica* and *S. haemastoma* [Stickle et al., 1989; Das and Stickle, 1993]. Reduction of metabolic rate upon exposure to hypoxia is a survival strategy utilized much more effectively by annelids and molluscs than by crustaceans [Gnaiger, 1983; Stickle et al., 1989]. Thus, it is not surprising that blue crabs have well-developed mechanisms for procuring oxygen.

Hypercapnic Hypoxia

As it does in aerial environments, photosynthesis fixes carbon dioxide in the aquatic environment, removing it from the water. However, gases are roughly 7,000 times less diffusible in water than in air [Dejours, 1975] and bodies of water are rarely homogeneous with respect to dissolved oxygen or carbon dioxide. In addition, the capacity of water to hold oxygen is significantly lower than that of air ($53.8 \mu\text{mol O}_2 \text{ l}^{-1} \text{ torr}^{-1}$ in air at 25°C as compared to $1.4 \mu\text{mol O}_2 \text{ l}^{-1} \text{ torr}^{-1}$ in sea water or $1.7 \mu\text{mol O}_2 \text{ l}^{-1} \text{ torr}^{-1}$ in fresh water). Water is able to hold more carbon dioxide in all its chemical forms than oxygen because of the hydration reactions of carbon dioxide that produce bicarbonate and carbonate ions. In estuaries the production of oxygen by photosynthesis can lead to oxygen pressures that are significantly higher than that of air [Atkinson et al., 1987]. However, during the night when photosynthesis is greatly reduced or absent, the respiratory consumption of oxygen and production of carbon dioxide results in water that is both hypoxic and hypercapnic. In the Chesapeake Bay, the bacterioplankton can account for 60 to 100% of the planktonic oxygen consumption, especially in water rich in dissolved organics [Jonas, 1997]. In shallow salt marshes, water can become hypoxic as well as hypercapnic [Cochran and Burnett, 1996].

Many investigators studying the effects of hypoxia on organisms have induced hypoxia experimentally by gassing the water with nitrogen. However, hypoxia in the field is perhaps most often accompanied by a slight, but significant elevation in water carbon dioxide pressure (hypercapnia), resulting in large decreases in water pH [Cochran and Burnett, 1996; Burnett, 1997]. Through this mechanism water pH is highly correlated with oxygen levels [Christmas and Jordan, 1987; Burnett, 1997]. Hypercapnia, independent of dissolved oxygen, can have dramatic effects on the physiology of marine organisms. An elevation of ambient CO_2 results in a concomitant elevation of CO_2 in the bodies of organisms. The direct result of hypercapnia is a decrease in the pH of tissues and body fluids, which can have profound effects on a number of functions. For example, respiratory pigments are highly sensitive to pH resulting in a decrease of oxygen affinity in most organisms, but as discussed below, some organisms have a separate CO_2 -specific effect that increases oxygen affinity counteracting the pH-specific effect. In a hypoxic environment, this would be maladaptive. In addition, hemocytes circulating in the blood may have a reduced protective function as a cellular defense mechanism (see below).

Again the blue crab, *Callinectes sapidus*, offers an example of an adaptive response to hypercapnic hypoxia. The hemocyanin of *C. sapidus* has a specific CO_2 effect [Mangum and Burnett, 1986] that contributes to an increase in oxygen affinity as CO_2 pressure increases. The CO_2 -specific effect on hemocyanin is opposite to the well-known pH-specific effect (the Bohr shift in which a decrease in pH reduces oxygen affinity). During hypercapnic hypoxia the production of lactate specifically increases hemocyanin oxygen affinity (a third and also separate effect) and this will also contribute to an adaptive response. Carbon dioxide also produces a large increase in hemocyanin oxygen affinity in the grass shrimp, *Palaemonetes pugio*, independent of pH. Cochran and Burnett [1996] have shown that moderate hypercapnia does not affect the ability of grass shrimp to regulate oxygen uptake during declining oxygen tensions. Thus, the presence of a CO_2 -specific effect brings about an adaptive increase in oxygen affinity. This stabilization of hemocyanin function in grass shrimp is thought to result in the maintenance in hypercapnic water of a "critical oxygen pressure," the point at which regulation of oxygen uptake ceases [Cochran and Burnett, 1996].

TABLE 2. Critical P_{O_2} and oxygen uptake at $P_{O_2} > 100$ torr in a variety of small coastal fishes collected in Charleston Harbor or on the ocean beach of Folly Beach, South Carolina. No distinction was made between male and female fish. Fishes were held at 25‰S and 30°C and subjected to declining oxygen using the methods of Cochran and Burnett [1996]. Critical P_{O_2} and O_2 Uptake are reported as mean (SEM; N).

Species	CO ₂ Treatment	Critical P_{O_2} (torr)	O ₂ Uptake ($\mu\text{mol g}^{-1} \text{h}^{-1}$)	Mean Wt. (g)
<i>Cyprinodon variegatus</i> sheepshead minnow	Low (< 1 torr)	17.1 (1.8; 8)	10.0 (0.7; 8)	1.67
	High (7 torr)	20.5 (4.1; 8)	11.8 (0.9; 8)	1.95
<i>Poecilia latipina</i> Sailfin molly	Low (< 1 torr)	37.5 (2.3; 6)	20.0 (1.6; 6)	1.01
	High (7 torr)	33.9 (4.0; 7)	12.5 (1.8; 7) ^b	1.16
<i>Trachinotus carolinus</i> Pompano	Low (< 1 torr)	32.5 (1.7; 8)	21.6 (2.4; 8)	8.10
	High (7 torr)	71.7 (9.2; 5) ^a	17.8 (1.4; 5)	4.86
<i>Mugil cephalus</i> mullet	Low (< 1 torr)	44.5 (5.4; 6)	53.5 (5.2; 6)	0.18
	High (7 torr)	36.4 (1.8; 7)	35.5 (2.8; 7) ^b	0.16

^asignificant difference ($p < 0.05$) from low CO₂ according to Mann-Whitney rank sum test.

^bsignificant difference ($p < 0.05$) from low CO₂ according to t-test.

Hypercapnia can have respiratory effects on fishes. The spot, *Leiostomus xanthurus*, showed a significantly elevated rate of oxygen uptake in mild ($P_{CO_2} = 7$ torr) hypercapnia [Cochran and Burnett, 1996]. The authors attributed this response to a Root effect in this species of fish [Bonaventura et al., 1976] that, under acidic environmental conditions, effectively ties up a portion of its hemoglobin preventing it from transporting oxygen and forcing the fish to use ventilatory and circulatory mechanisms to extract oxygen from the water. Increasing ventilation and circulation requires more metabolic energy, thus elevating oxygen uptake. On the other hand, the mummichog, *Fundulus heteroclitus*, was not affected by similar mild hypercapnia [Cochran and Burnett, 1996].

Using methods identical to those of Cochran and Burnett [1996] the responses of small coastal fishes to hypercapnic hypoxia were investigated (Table 2). Fish were held in respirometers for several hours and the respirometers were flushed with well-oxygenated water, until oxygen uptake by fish declined to steady levels. Respirometers were then closed and the oxygen pressures allowed to decline as fish consumed oxygen. Carbon dioxide within the respirometer was regulated by monitoring its level within the respirometer and periodically flushing the chamber with water of identical oxygen pressure (i.e., when the flushing began) and either high (1%) or low (< 0.1%) carbon dioxide, depending upon the treatment. The pompano, *Trachinotus carolinus*, had a critical P_{O_2} that was significantly elevated in mild hypercapnia, a result not surprising for a species that inhabits the well oxygenated waters of the inlets and the beaches of coastal South Carolina where it rarely encounters hypercapnic hypoxia. The critical P_{O_2} of the other species was insensitive to mild hypercapnia. Only *Poecilia latipina* and *Mugil cephalus* showed a significant depression in oxygen uptake at high P_{O_2} and mild hypercapnia (Table 2). These results are

unlike those of *L. xanthurus* (above) and may be due to different mechanisms that reduce metabolism. A similar depression of metabolism by hypercapnia and the concomitant decline in pH has been documented in trout hepatocytes [Walsh et al., 1988]. The mullet (*M. cephalus*) used in the study had a very high rate of oxygen uptake at low CO₂ pressures. Mullet are a very active fish and it is difficult to obtain "resting" oxygen uptake rates within a closed respirometer. *Cyprinodon variegatus*, the most hardy of the fishes listed in Table 2, is commonly found in estuarine environments where hypercapnic hypoxia occurs frequently. It would be interesting to know the responses of these fishes to more severe hypercapnia.

Disease Resistance

The effects of environmental hypoxia on the cellular defenses of aquatic organisms is largely unknown, but is an emerging field of interest. The data that exist suggest that hypoxia can have profound effects on immune systems. This area of research has much relevance to organisms that are cultured for commercial purposes, where organism densities are high and the potential for pathogenic infections is great.

Hemocytes in invertebrates are responsible for the phagocytosis of potential pathogens as a part of an innate immune response. There is evidence that phagocytic activity in shrimp is depressed when the shrimp (*Penaeus monodon*) are exposed to hypoxia [Direkbusarakom and Danayadol, 1998]. Total hemocyte numbers can also be influenced by hypoxia. Le Moullac et al. [1998] have shown that shrimp (*Penaeus stylirostris*) exposed to severe hypoxia (1 mg O₂ l⁻¹ = 25 torr = 16% air saturation) had a decrease in total hemocyte count. These effects appear to be relevant, as it was shown that shrimp injected with the pathogenic *Vibrio alginolyticus* showed significantly greater mortality when exposed to hypoxia [Le Moullac et al., 1998]

Hypoxia negatively impacts the production of reactive oxygen species by hemocytes of the oyster *Crassostrea virginica* [Boyd and Burnett, 1999]. In this case, oyster hemocytes incubated at the physiological O₂ and CO₂ pressures that exist in oyster hemolymph during moderate hypercapnic hypoxia (P_{O₂} = 40 to 45 torr) produced only 33% of the reactive oxygen species compared with normoxic conditions. Furthermore, this result was shown to be due to the separate and independent influences of oxygen (64% of normoxia) and pH (44% of normoxia). This study points to the importance of performing *in vitro* experiments at the physiological gas pressures that components of the immune system experience *in vivo*.

Future Directions

One final point deserves mention because it is often overlooked in discussions of the effects of hypoxia on organisms. Other environmental factors can induce indirectly an "internal" hypoxia in organisms. For example, bivalved molluscs can close themselves from the aquatic environment when conditions are unfavorable, e.g., when salinity changes rapidly or when they are disturbed by a predator. A closed bivalve does not exchange gas with the environment and tissue oxygen levels decline [Crenshaw and Neff, 1969]. The same thing can happen during air exposure when the shells of a bivalve close or when the

gills of a water breather collapse. Anaerobic metabolism can predominate when tissues become hypoxic. The polychaete annelid, *Arenicola marina*, enters anaerobiosis when ambient temperature is 4°C above the aerobic threshold boundary temperature [Sommer et al., 1997; Sommer and Pörtner, 1999]. The sea urchin, *Strongylocentrotus droebachiensis*, switches to anaerobic metabolism and produces lactic acid when the coelomic cavity is full with ripe gonads [Bookbinder and Shick, 1986]; lactic acid concentrations also increase in the coelomic cavity of ripe sea urchins as environmental salinity decreases [Roller and Stickle, 1994]. These types of hypoxia may be indistinguishable, from a physiological perspective, from that induced by ambient environmental hypoxia and they should not be ignored.

As hypoxic water becomes a greater threat to coastal habitats [Diaz and Rosenberg, 1995], the need to know how low oxygen affects organisms becomes more important. There is a need for greater understanding of the sublethal effects of hypoxia on individual organisms and how these effects influence population densities and distributions. Studies done carefully on the influence of oxygen on the growth, the feeding and the predation behavior of organisms will help us to understand better the results of hypoxia at the population level. It is also important to distinguish between the acute responses and the chronic responses of an organism to hypoxia. Diurnal fluctuations in oxygen can produce responses different from chronic exposures. Investigators should also be aware that the hypercapnic and low pH water that accompanies hypoxia can have separate and profound negative effects on organisms. Finally, the immune responses of organisms appear to be sensitive to low oxygen. Studies of the components of the immune systems of aquatic organisms are needed that take into account the gas pressures and the physiological conditions that occur *in vivo*.

Acknowledgements. This work was supported in part by a grant to L. Burnett from the Charleston Harbor Project and SC Sea Grant R/ER-14. Contribution 163 of the Grice Marine Laboratory.

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