

## The Challenges of Living in Hypoxic and Hypercapnic Aquatic Environments<sup>1,2</sup>

LOUIS E. BURNETT<sup>3</sup>

Grice Marine Biological Laboratory University of Charleston, South Carolina and  
205 Fort Johnson, Charleston, South Carolina 29412

**SYNOPSIS.** Organisms living in coastal waters, and especially estuaries, have long been known to have behavioral or physiological mechanisms that enable them to exist in water containing low amounts of oxygen. However, the respiratory consumption of oxygen that generates hypoxia is also responsible for producing significant amounts of carbon dioxide. An elevation of carbon dioxide pressure in water will cause a significant acidosis in most aquatic organisms. Thus, the combination of low oxygen and elevated carbon dioxide that occurs in estuaries represents a significant environmental challenge to organisms living in this habitat. Organisms may maintain oxygen uptake in declining oxygen conditions by using a respiratory pigment and/or by making adjustments in the convective flow of water and blood past respiratory surfaces (*i.e.*, increase cardiac output and ventilation rate). Severe hypoxia may result in an organism switching partially or completely to anaerobic biochemical pathways to sustain metabolic rate. There is also evidence to suggest that organisms lower their metabolism during hypoxic stress. Elevated water CO<sub>2</sub> (hypercapnia) produces an acidosis in the tissues of organisms that breathe it. This acidosis may be wholly or partially compensated (*i.e.*, mechanisms return pH to pre-exposure levels), or may be uncompensated. Some studies have examined the effects on organisms of exposure simultaneously to hypoxia and hypercapnia. This article reviews some of the specific adaptations and responses of organisms to low oxygen, to high carbon dioxide, and to the co-occurrence of low oxygen and high carbon dioxide.

### INTRODUCTION

A significant factor in limiting the distribution of macroorganisms in aquatic environments is the availability of oxygen. This is true because nearly all animals require oxygen in the process of producing energy. Estuaries are particularly noted for the development of low environmental oxygen levels (hypoxia), although they are not unique in this respect (Diaz and Rosenberg, 1995). Water-breathing animals that live in estuaries may experience extremes in oxygen availability (*e.g.*, Renaud, 1985; Atkinson *et al.*, 1987; Rabalais *et al.*, 1994). This appears to be a part of a cycle that is seasonal and tidal in the estuaries associated

with salt marshes along the southeastern United States (Cochran and Burnett, 1996).

An estuary is defined as a semi-enclosed body of water with freshwater input and tidal flushing by the ocean (Cameron and Pritchard, 1963). Because of the water runoff from the land, estuaries are rich in nutrients and are typically considered highly productive environments. Thus, as a fisheries resource, estuaries are important. However, because coastal areas sustain large human populations, the impacts of human activity on estuarine ecosystems can be quite profound. Diaz and Rosenberg (1995), in a review of the ecological effects of hypoxia, conclude that human activities have enhanced the occurrence of hypoxia and anoxia in a number of important ecosystems around the world, including estuaries. These authors suggest that many ecosystems are now severely stressed by hypoxia.

In this article I will review the effects of hypoxia and hypercapnia (high CO<sub>2</sub>) on selected estuarine organisms. I will focus on

<sup>1</sup> From the Symposium *Molecules to Mudflats* presented at the Annual Meeting of the Society for Integrative and Comparative Biology, 26-30 December 1995, at Washington, D.C.

<sup>2</sup> Contribution No. 137 of the Grice Marine Biological Laboratory.

<sup>3</sup> E-mail: burnettl@cofc.edu.

estuarine organisms because much work on organisms has centered on estuaries. However, the information presented here is applicable to any organism encountering hypoxia and/or hypercapnia. There is a large literature on organismal adaptations to hypoxia and I will not attempt to summarize that literature here. Rather, I will provide an overview of the strategies different organisms have used to live in hypoxic environments. Much less has been done on adaptations to hypercapnia and to combinations of hypoxia and hypercapnia.

It is well-known that many organisms are able to resist and/or compensate for low levels of environmental oxygen (Mangum and Van Winkle, 1973; Grieshaber *et al.*, 1994; Mangum, 1997 this symposium). However, it is not well appreciated that environmental hypoxia is nearly always accompanied by an elevation of water carbon dioxide (and thus a decrease in water pH). The biological oxygen demand responsible for lowering oxygen levels produces carbon dioxide as the main product of metabolism. The same processes occur in aerial environments. As it does in aerial environments, photosynthesis in water fixes carbon dioxide, removing it from the water. However, gases are roughly 7,000 times less diffusible in water than air (Dejours, 1975). Because gases are not very mobile in water, bodies of water are rarely homogeneous with respect to dissolved oxygen or carbon dioxide. In addition, the capacity of water to hold molecules of oxygen is significantly lower than that of air ( $53.8 \mu\text{mol Liter}^{-1} \text{ torr}^{-1}$  in air at  $25^\circ\text{C}$  as compared to  $1.4 \mu\text{mol liter}^{-1} \text{ torr}^{-1}$  in sea water or  $1.7 \mu\text{mol liter}^{-1} \text{ torr}^{-1}$  in fresh water). Water is able to hold more carbon dioxide than oxygen because of the hydration reactions of carbon dioxide that produce bicarbonate and carbonate ions.

Photosynthesis removes carbon dioxide from the water and produces oxygen. In estuaries the production of oxygen can lead to oxygen pressures that are significantly higher than that of air (Atkinson *et al.*, 1987). However, during the night photosynthesis does not occur and respiratory consumption of oxygen and production of carbon dioxide results in water that is hypoxic

and hypercapnic. Furthermore, in some waters, such as 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). Even in shallow salt marshes, water can become hypoxic as well as hypercapnic (Cochran and Burnett, 1996). Along the Southeastern coast of the United States there are extensive salt marshes. In the coastal areas around Charleston, South Carolina approximately 80% of the area between mean high water and mean low water consists of salt marshes covered primarily by *Spartina alterniflora*. In this region the tidal amplitude is about 2 m. Water draining from the marsh on an ebbing tide is nearly always hypoxic and has a low pH (see Cochran and Burnett, 1996). Although this phenomenon occurs on ebbing tides in the daylight and the dark, the hypoxia and the low pH are more severe during the dark. These results suggest that significant oxygen consumption is occurring in the salt marshes.

The correlation between the amount of oxygen in the water and the pH of water is very striking (Fig. 1 and see Christmas and Jordan, 1987), although water pH is not affected directly by oxygen. The high correlation suggests and is consistent with a functional link between the consumption of oxygen and the production of carbon dioxide, *i.e.*, organisms consume oxygen, reducing oxygen levels and produce carbon dioxide, lowering pH. Water pH can also be influenced by runoff of strong and weak acids and bases into tidal creeks, but these mechanisms would not necessarily be tightly correlated with oxygen. Oxygen and pH, in some instances, can change very rapidly and can be tightly correlated to tidal fluctuations (Holland *et al.*, 1996).

Salt marshes are important to the high productivity of these estuaries because they provide an environment where key species come to feed. In addition, because the environmental conditions of the salt marsh are considered severe, this habitat may serve as a sanctuary protecting the feeding organisms from predators that are less adapted to live in this area.

Scientists have documented many mor-

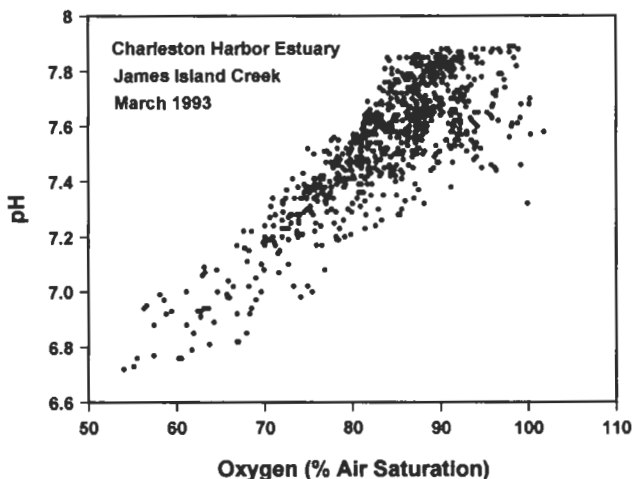


FIG. 1. Oxygen and pH in water samples taken from James Island Creek in the Charleston Harbor Estuary in March 1993. The high correlation between these two variables suggests that changes in pH are explained largely by respiratory production or photosynthetic consumption of  $\text{CO}_2$ . Data provided by Phillip Dustan, College of Charleston from work supported by the Charleston Harbor Project.

phological, physiological, behavioral, and molecular adaptations that organisms possess to deal with hypoxia and anoxia (see Grieshaber *et al.*, 1994 for review). Some studies describe the effects of environmental hypercapnia (Cameron, 1976, 1978; Heisler, 1982; Lindinger *et al.*, 1984). However, the effects of hypoxia combined with hypercapnia have largely been undescribed. This combination is important because the two gases have profound and independent effects on the physiology of estuarine organisms.

#### HYPOXIA

There is an extensive literature on organismal responses to hypoxia. The first lines of defense are behavioral mechanisms that can be used. Clearly, mobile organisms can move to a different location. Cochran and Burnett (1996) suggested that juvenile spot, *Leiostomus xanthurus*, while tolerant of fairly hypoxic water (25% air saturation), avoid severely hypoxic water as has been shown in other fishes (Dandy, 1970; Spoor, 1990) and penaeid shrimp (Renaud, 1986). Animals can take advantage of microhabitats that are richer in oxygen. The killifish, *Fundulus heteroclitus*, is morphologically adapted (Lewis, 1970), as are other fishes (Gee *et al.*, 1978), to take advantage of the

abundance of oxygen at the surface of the water (Kramer and Mehegen, 1981). The grass shrimp, *Palaemonetes pugio*, is an important estuarine species that may solve the problem by escaping periodically to air, literally jumping out of the water, where it can take advantage of a rich supply of oxygen (Welsh, 1975; Cochran and Burnett, 1996).

If animals are confined to a hypoxic zone, however, they must either compensate for decreases in ambient oxygen to maintain their metabolism, lower their demand for energy, or use alternative methods of producing energy that do not require oxygen. Combinations of these three mechanisms are known to exist.

#### *Maintaining aerobic metabolism during hypoxia*

Many animals can maintain oxygen uptake in declining environmental oxygen by using a variety of respiratory adaptations including increasing heart rate and increasing the ventilatory flow of water past the respiratory surfaces (*e.g.*, McMahan and Wilkens, 1983; Cameron, 1989). Organisms with respiratory pigments have additional mechanisms at their disposal to optimize the uptake of oxygen. Respiratory pigments can be particularly effective at maximizing

oxygen pressure gradients between the ambient environment and the blood or hemolymph at the respiratory surfaces, thereby maximizing the flux of oxygen. It has long been known that respiratory pigments in some organisms are inducible, *i.e.*, the production of pigment is stimulated by hypoxia (Mangum, 1997 this symposium). In addition, hypoxia stimulates structural changes in the hemocyanin of blue crabs *Callinectes sapidus* resulting in a pigment with a higher oxygen affinity and, therefore, better suited to a hypoxic environment (deFur *et al.*, 1990).

Some estuarine animals are nearly perfect regulators of oxygen uptake (Cochran and Burnett, 1996), *i.e.*, they can maintain constant rates of oxygen uptake as the oxygen levels in the ambient medium decline. However, at some point, called the critical oxygen pressure, the animal is no longer able to sustain oxygen uptake and uptake declines with oxygen pressure. At this point the organism may switch to using anaerobic metabolism to sustain part of its energy needs. Cochran and Burnett (1996) measured oxygen uptake as a function of environmental oxygen in three estuarine species that occupy the salt marsh. Remarkable similarities were found among the three species in that all regulated oxygen uptake down to oxygen pressures of between 34 and 42 torr (22 to 27% of air saturation). Each of the three species produced significant amounts of lactate, a product of anaerobic metabolism, when ambient oxygen pressures fell below the critical pressure. While many organisms may survive brief periods below critical oxygen pressures, their survival appears to be sensitive to the duration of exposure (Cochran and Burnett, 1996).

#### *Decreasing metabolism during hypoxia*

Many estuarine organisms are well known for their ability to use anaerobic pathways to produce energy. However, the ability of organisms to reduce their aerobic energy demand in response to environmental changes other than temperature has not been studied extensively. Investigations of intertidal bivalves using direct calorimetry and the appearance of anaerobic endprod-

ucts reveal that the overall metabolic rates of some bivalves decrease dramatically when exposed to air or nitrogen atmospheres (Widdows *et al.*, 1979; Shick *et al.*, 1983; Widdows and Shick, 1985; Shick *et al.*, 1986). Burnett and McMahon (1987) presented evidence suggesting that the intertidal mud crab *Eurytium albidigitum* responds to air exposure by lowering its aerobic metabolism. In these cases, air exposure is comparable to hypoxia in that unless an organism has specific morphological or physiological adaptations for breathing air instead of water, it may not have access to the rich supply of oxygen in the aerial environment. Thus, tissues may become hypoxic during air exposure. Widdows *et al.* (1989) have shown that oyster larvae can respond to hypoxia by lowering metabolism. These authors also found that the feeding rates of oyster larvae are also depressed during hypoxia. Metabolic responses to hypoxia have been reviewed recently by Grieshaber *et al.* (1994).

#### HYPERCAPNIA

Elevated carbon dioxide pressures in the water (hypercapnia) produce an acidosis in the blood of organisms that is respiratory in nature, *i.e.*, the acidosis occurs due to elevated blood  $PCO_2$ . As the  $PCO_2$  in water rises, the  $PCO_2$  in tissues of animals in the water also rises. This leads



to an elevation of both bicarbonate ions and hydrogen ions in the ambient water as well as in the tissues. Hypercapnia has been studied in some estuarine organisms primarily as a means to learn how organisms respond to acid-base disturbances (*e.g.*, Cameron, 1978; Lindinger *et al.*, 1984). Crustaceans and fishes compensate partially for a  $CO_2$ -induced acidosis by elevating blood bicarbonate ion levels (Cameron, 1976, 1978; Heisler, 1982). These changes are thought to be brought about by ionic exchanges between the blood and the ambient environment. Bivalve molluscs also compensate partially for a hypercapnia-induced acidosis (Lindinger *et al.*, 1984). Compensation in molluscs appears to be brought about primarily by elevated he-

molymph calcium ion and ammonium concentrations (Booth *et al.*, 1984; Lindinger *et al.*, 1984; Dwyer and Burnett, 1996). Calcium ions are thought to be generated by dissolving the heavily calcified shell (Crenshaw and Neff, 1969).

In all cases mentioned above compensation is not complete. In other words, hemolymph or blood pH is not completely restored to the values present before the hypercapnic challenge. The degree of compensation depends upon the magnitude of the challenge. The compensation that does occur takes several hours. The result is that estuarine organisms challenged with elevated environmental  $\text{PCO}_2$  sustain an acidosis to varying degrees for the duration of the exposure. Some of the physiological implications of tissue hypercapnia and the ensuing acidosis are discussed in the section below.

#### HYPOXIA AND HYPERCAPNIA

As emphasized above, environmental hypoxia and hypercapnia co-occur commonly in estuarine environments. The mechanisms an organism uses to meet the challenge of low environmental oxygen must be used while it is also experiencing a rapid and system-wide acidosis induced by elevated water  $\text{PCO}_2$ . The killifish, *Fundulus heteroclitus*, juvenile spot, *Leiostomus xanthurus*, and the grass shrimp, *Palaemonetes pugio*, are strong regulators of oxygen uptake in declining ambient oxygen (Cochran and Burnett, 1996). More severe hypoxia results in a decline in oxygen uptake and each of these organisms accumulates lactic acid indicating that they are using anaerobic pathways to produce energy. The critical oxygen pressure ( $P_{\text{crit}}$ ) in each of the three organisms is unaffected when declining oxygen occurs simultaneously with hypercapnia. In fact, *P. pugio* possesses the respiratory pigment hemocyanin that increases its affinity for oxygen when  $\text{PCO}_2$  rises (Mangum and Burnett, 1986). The increase in oxygen affinity is a specific effect of  $\text{CO}_2$ , independent of pH, and is clearly an adaptive response.

The response of the blue crab *Callinectes sapidus* to hypoxia has been well-studied. deFur *et al.* (1990) held blue crabs in hyp-

oxic (but not hypercapnic) water for 7–25 days and observed a number of adaptive responses including an increase in hemocyanin concentration and an increase in hemocyanin oxygen affinity brought about largely by structural changes within the pigment. Peter deFur and I (deFur and Burnett, 1995) have investigated the influence of exposure to hypoxia and hypercapnia simultaneously in the blue crab *Callinectes sapidus*. When crabs are held in aquariums at 25°C and 25 ppt salinity and oxygen levels are allowed to drop due to the uptake of oxygen by the crabs, water  $\text{PCO}_2$  rises. This situation is similar to that which occurs in the natural environment. In moderate hypoxia (58% air saturation) and hypercapnia ( $\text{PCO}_2 = 2.6$  torr) venous hemolymph  $\text{PO}_2$  remains unchanged (8 torr) while venous  $\text{PCO}_2$  rises (2.8 to 5 torr). However, hemolymph pH stays the same (pH = 7.6). In more severe hypoxia (12% air saturation) and somewhat greater hypercapnia ( $\text{PCO}_2 = 3.7$  torr), venous hemolymph  $\text{PCO}_2$  decreases to very low levels (1.6 torr) while  $\text{PCO}_2$  remains unchanged (5 torr) compared to values at moderate hypoxia. Hemolymph pH rises significantly (pH = 7.76) as does total hemolymph  $\text{CO}_2$  (from 6.4 to 13.7  $\text{mmol liter}^{-1}$ ) and lactate concentration (from 1 to 2.1  $\text{mmol liter}^{-1}$ ). The increase in pH is adaptive because it causes a significant increase in hemocyanin oxygen affinity (Booth *et al.*, 1982). Furthermore, the hemocyanin of *C. sapidus* has a specific  $\text{CO}_2$  effect (Mangum and Burnett, 1986) and a lactate effect (Booth *et al.*, 1982), both of which contribute to an increase in oxygen affinity as  $\text{CO}_2$  and lactate increase. The mechanisms of the increase in hemolymph pH are unknown but the large rise in hemolymph bicarbonate concentration (deFur and Burnett, 1995) suggests that compensation is, in the jargon of acid-base physiology, “metabolic” in nature. This may take the form of an elevation of hemolymph calcium ion concentrations or changes in the concentrations of strong ions such as sodium and chloride in the hemolymph to increase the strong ion difference. The slight increase in lactate during severe hypoxia/hypercapnia indicates that the blue

crab is, in part, relying on anaerobic metabolism.

My students and I have recently focused on the effects of hypoxia and hypercapnia on oyster physiology. Dwyer and Burnett (1996) have reported that infections of the Easter oyster, *Crassostrea virginica*, with the protozoan parasite *Perkinsus marinus* induce a significant hemolymph acidosis. Dwyer and Burnett (1996) have suggested that acidic conditions favor the growth of *P. marinus* within the oyster and may also stimulate the spread of *P. marinus*. Boyd and Burnett (1995) have reported preliminary evidence showing that reactive oxygen intermediate (ROI) production by oyster hemocytes is greatly reduced when hemocytes are held under the  $PO_2$  and  $PCO_2$  mimicking levels in the hemolymph of oysters held under environmental hypoxia and hypercapnia. Reactive oxygen intermediates such as hydrogen peroxide ( $H_2O_2$ ), superoxide ( $O_2^-$ ) and hypochlorous acid (HOCl) are produced by oyster hemocytes to destroy invading foreign cells. These ROIs can damage invading cells in a variety of ways, including DNA breakage, enzyme inhibition, degradation of membrane integrity, reaction with unsaturated lipids and the yielding of toxic unsaturated fatty acid aldehydes and alkyl radicals (Adema *et al.*, 1991). Furthermore, ROI production appears to be highly sensitive to  $PCO_2$ . We do not yet know if the sensitivity is a specific effect of increased  $CO_2$  or the resulting decline in pH. This result is important because ROI production is considered to be a significant line of defense in the oyster against foreign substances (Anderson, 1996). Oysters breathing water that is low in oxygen and high in carbon dioxide may, therefore, be especially vulnerable to infection by parasites such as *P. marinus*. While oysters may be well-adapted physiologically to live in water in which they are stressed with hypoxia and hypercapnia, these environmental variables stimulate parasitism and at the same time lower the defense mechanisms against parasites. The co-occurrence of hypoxia and hypercapnia may have contributed to the decline in oyster populations where dissolved oxygen concentrations are known to be low. Christmas and Jordan

(1987) have documented a strong positive correlation between dissolved oxygen and pH on six Choptank River oyster bars in Maryland. The correlation of oxygen and pH (discussed earlier) in this case suggests that the water was also hypercapnic.

There is little information available to suggest how organisms respond to stresses associated with contaminants when they are also challenged with hypercapnia and hypoxia. A particular contaminant may also influence an organism indirectly. For example, an herbicide such as atrazine, which is a photosynthesis inhibitor commonly applied to crops as well as residential lawns, inhibits the growth of nanophytoplankton in estuaries (Pennington, 1996). This could curtail the uptake of carbon dioxide and production of oxygen in the water column, a condition leading to more severe hypoxia and hypercapnia. While low levels of atrazine are not known to affect estuarine animals directly, atrazine could contribute indirectly to environmental conditions that enhance stress.

It is clear from the above examples that a number of estuarine organisms have mechanisms to respond to hypoxia and hypercapnia in the environment. However, we do not know how these organisms might respond to bouts of hypercapnic hypoxia that are more extreme, are of longer duration, or that occur simultaneously with changes in temperature and salinity. Investigators studying the effects of environmental contaminants on the biology of individual species should design experiments to mimic conditions that the organisms experience in their natural habitats. These should include the occurrence simultaneously of hypoxia and hypercapnia and the occurrence of parasites.

#### ACKNOWLEDGMENTS

The work on this manuscript was done while the author was supported by grants from the Charleston Harbor Project, the South Carolina Sea Grant Consortium, and the Oyster Disease Research Program, NOAA (NA47FL0151).

#### REFERENCES

- Adema, C. M., W. P. W. Van der Knaap, and T. Sminia. 1991. Molluscan hemocyte-mediated cytotoxicity

ty: The role of reactive oxygen intermediates. Reviews in Aquatic Sciences, 4:201-223.

- Anderson, R. S. 1996. Interactions of *Perkinsus marinus* with humoral factors and hemocytes of *Crassostrea virginica*. J. Shellfish Res. 15:127-134.
- Atkinson, M. J., T. Berman, B. R. Allanson, and J. Imberger. 1987. Fine-scale oxygen availability in a stratified estuary: Patchiness in aquatic environments. Mar. Ecol. Prog. Ser. 36:1-10.
- Booth, C. E., B. R. McMahon, and A. W. Pinder. 1982. Oxygen uptake and the potentiating effects of increased hemolymph lactate on oxygen transport during exercise in the blue crab *Callinectes sapidus*. J. Comp. Physiol. 148:111-121.
- Booth, C. E., D. G. McDonald, and P. J. Walsh. 1984. Acid-base balance in the sea mussel, *Mytilus edulis*. I. Effects of hypoxia and air-exposure on hemolymph acid-base status. Mar. Biol. Lett. 5:347-358.
- Boyd, J. N. and L. E. Burnett. 1995. Reactive oxygen intermediate production in oyster hemocytes exposed to hypoxia. Amer. Zool. 35:33A.
- Burnett, L. E. and B. R. McMahon. 1987. O<sub>2</sub> uptake, acid-base balance and branchial water CO<sub>2</sub> content during air exposure in intertidal crabs. Physiol. Zool. 60:27-36.
- Cameron, J. N. 1976. Branchial ion uptake in arctic grayling: Resting values and effects of acid-base disturbance. J. Exp. Biol. 64:711-725.
- Cameron, J. N. 1978. Effects of hypercapnia on blood acid-base status, NaCl fluxes, and transgill potential in freshwater blue crabs, *Callinectes sapidus*. J. Comp. Physiol. 123:137-141.
- Cameron, J. N. 1989. *The respiratory physiology of animals*. Oxford University Press. New York. 353 pp.
- Cameron, W. M. and D. W. Pritchard. 1963. Estuaries. In M. N. Hill (ed.), *The sea: Ideas and observations*, Vol. 2, Ch. 15, pp. 306-324. Wiley-Interscience.
- Christmas, J. F. and S. J. Jordan. 1987. Biological monitoring of selected oyster bars in the lower Choptank. In G. B. MacKiernan, (ed.), *Dissolved oxygen in the Chesapeake Bay*, pp. 125-128. Maryland Sea Grant, College Park, Maryland, No. UM-SG-TS-87-03.
- Cochran, R. E. and L. E. Burnett. 1996. Respiratory responses of the salt marsh animals, *Fundulus heteroclitus*, *Leiostomus xanthurus*, and *Palaemonetes pugio* to environmental hypoxia and hypercapnia and to the organophosphate pesticide, azinphosmethyl. J. Exp. Mar. Biol. Ecol. 195:125-144.
- Crenshaw, M. A. and J. M. Neff. 1969. Decalcification at the mantle-shell interface in mollusks. Amer. Zool. 9:881-889.
- Dandy, J. W. 1970. Activity responses to oxygen in the brook trout, *Salvelinus fontinalis*. Can J. Zool. 48:1067-1072.
- deFur, P. L. and L. E. Burnett. 1995. Exposure to hypercapnic hypoxia alters respiratory function in blue crabs, *Callinectes sapidus*. Amer. Zool. 35:66A.
- deFur, P. L., C. P. Mangum, and J. E. Reese. 1990. Respiratory responses of the blue crab *Callinectes sapidus* to long-term hypoxia. Biol. Bull. 178:46-54.
- Dejours, P. 1975. *Principles of comparative respiratory physiology*. American Elsevier Publishing Company, Inc., New York.
- Diaz, R. J. and R. Rosenburg. 1995. Marine benthic hypoxia: A review of its ecological effects and the behavioral responses of benthic macrofauna. Oceanography and Marine Biology Annual Review 33:245-303.
- Dwyer, J. J. and L. E. Burnett. 1996. Acid-base status of the oyster *Crassostrea virginica* in response to air exposure and to infections by *Perkinsus marinus*. Biol. Bull. 190:139-147.
- Gee, J. H., R. F. Tallman, and H. J. Smart. 1978. Reactions of some great plains fishes to progressive hypoxia. Can. J. Zool. 56:1962-1966.
- Grieshaber, M. K., I. Hardewig, U. Kreutzer, and H.-O. Pörtner. 1994. Physiological and metabolic responses to hypoxia in invertebrates. Rev. Physiol. Biochem. Pharmacol. 125:44-147.
- Heisler, N. 1982. Transepithelial ion transfer processes as mechanisms for fish acid-base regulation in hypercapnia and lactacidosis. Can. J. Zool. 60:1108-1122.
- Holland, A. F., G. H. M. Riekerk, S. B. Lerberg, L. E. Zimmerman, D. M. Sanger, T. D. Matthews, G. I. Scott, M. H. Fulton, B. C. Thompson, J. W. Dugomah, J. D. DeVane, K. M. Beck, and A. R. Diaz. 1996. The Tidal Creek Project, Interim Report. Charleston Harbor Project.
- Jonas, R. B. 1997. Bacteria, dissolved organics and oxygen consumption in salinity stratified Chesapeake Bay, an anoxia paradigm. Amer. Zool. 37:612-620.
- Kramer, D. L. and J. P. Mehegan. 1981. Aquatic surface respiration, an adaptive response to hypoxia in the guppy, *Poecilia reticulata* (Pisces, Poeciliidae). Env. Biol. Fish. 6:299-313.
- Lewis, W. M. Jr. 1970. Morphological adaptations of Cyprinodontoids for inhabiting oxygen deficient waters. Copeia 2:319-325.
- Lindinger, M. I., D. J. Laurén, and D. G. McDonald. 1984. Acid-base balance in the sea mussel, *Mytilus edulis*. III. Effects of environmental hypercapnia on intra- and extracellular acid-base balance. Mar. Biol. Lett. 5:371-381.
- Mangum, C. P. 1997. Adaptation of the oxygen transport system to hypoxia in the blue crab, *Callinectes sapidus*. Amer. Zool. 37:000-000.
- Mangum, C. and W. Van Winkle. 1973. Responses of aquatic invertebrates to declining oxygen conditions. Amer. Zool. 13:529-541.
- Mangum, C. P. and L. E. Burnett. 1986. The CO<sub>2</sub> sensitivity of the hemocyanin and its relationship to Cl<sup>-</sup> sensitivity. Biol. Bull. 171:248-263.
- McMahon, B. R. and J. L. Wilkens. 1983. Ventilation, perfusion, and oxygen uptake. In L. H. Mantel (ed.), *The biology of Crustacea*, pp. 289-372. Academic Press, New York.
- Officer, C. B., R. B. Biggs, J. L. Taft, L. E. Cronin, M. A. Tyler, and W. R. Boynton. 1984. Chesapeake Bay hypoxia: A review of its ecological effects and the behavioral responses of benthic macrofauna. Oceanography and Marine Biology Annual Review 33:245-303.

- peake Bay anoxia: Origin, development, and significance. *Science* 223:22-27.
- Pennington, P. L. 1996. The toxicity of the herbicides atrazine and alachlor on the estuarine phytoplankter *Pavlova* sp. (Prymnesiophyceae) with an emphasis on acute toxicity testing of individual herbicides, herbicide mixtures and multi-generational chronic bioassays. Master's Thesis, University of Charleston, Charleston South Carolina 140 pp.
- Rabalais, N. N., W. J. Wiseman, and R. E. Turner. 1994. Comparison of continuous records of near-bottom dissolved oxygen from the hypoxia zone along the Louisiana coast. *Estuaries* 17:850-861.
- Renaud, M. L. 1985. Hypoxia in Louisiana coastal waters during 1983: Implications for fisheries. *Fish. Bull.* 84:19-26.
- Renaud, M. L. 1986. Detecting and avoiding oxygen deficient sea water by brown shrimp, *Penaeus aztecus* (Ives), and white shrimp *Penaeus setiferus* (Linnaeus). *J. Exp. Mar. Biol. Ecol.* 98:283-292.
- Shick, J. M., A. de Zwaan and A. M. Th. de Bont. 1983. Anoxic metabolic rate in the mussel *Mytilus edulis* L. estimated by simultaneous direct calorimetry and biochemical analysis. *Physiol. Zool.* 56:56-63.
- Shick, J. M., E. Gnaiger, J. Widdows, B. L. Bayne and A. de Zwaan. 1986. Activity and metabolism in the mussel *Mytilus edulis* L. during intertidal hypoxia and aerobic recovery. *Physiol. Zool.* 59:627-642.
- Spoor, W. A. 1990. Distribution of fingerling brook trout, *Salvelinus fontinalis* (Mitchill), in dissolved oxygen concentration gradients. *J. Fish. Biol.* 36:363-373.
- Welsh, B. L. 1975. The role of grass shrimp, *Palaeomonetes pugio*, in a tidal marsh ecosystem. *Ecology* 56:513-530.
- Widdows, J., B. L. Bayne, D. R. Livingstone, R. I. E. Newell, and P. Donkin. 1979. Physiological and biochemical responses of bivalve molluscs to exposure to air. *Comp. Biochem. Physiol.* 62A:301-308.
- Widdows, J. and J. M. Shick. 1985. Physiological responses of *Mytilus edulis* and *Cardium edule* to aerial exposure. *Mar. Biol.* 85:217-232.
- Widdows, J., R. I. E. Newell, and R. Mann. 1989. Effects of hypoxia and anoxia on survival, energy metabolism, and feeding of oyster larvae (*Crassostrea virginica*, Gmelin). *Biol. Bull.* 177:154-166.